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(54) Title: CONSTRUCTION AND USE OF GENES ENCODING PATHOGENIC EPITOPES FOR TREATMENT OF AUTOIMMUNE DISEASE

(57) Abstract

The present invention relates to the application of genetic engineering to provide a treatment of autoimmune disease. This is achieved preferably through the introduction of one or more recombinant genes encoding self antigens which are the target of an autoimmune response. In particular the invention provides a method of designing and constructing a gene encoding an encephalitogenic epitope of proteolipid protein, and to the *in vivo* expression of the gene product by a recombinant retroviral vector. The expression and secretion of the encephalitogenic epitope ameliorates the histophathological and clinical characteristics of experimental autoimmune encephalomyelitis (EAE) in the mouse model for multiple sclerosis (MS).

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SPECIFICATION

CONSTRUCTION AND USE OF GENES ENCODING PATHOGENIC EPITOPES FOR TREATMENT OF AUTOIMMUNE DISEASE

Field of the Invention

This invention relates generally to the field of immunotherapy and to the preparation and use of engineered cells having the ability to restore tolerance to self antigens in patients suffering from autoimmune disease. More particularly, this invention relates to the design and construction of a gene encoding an encephalitogenic epitope of proteolipid protein (PLP), to methods of *in vitro* and *in vivo* expression of a PLP epitope, to methods of in vivo secretion of a PLP epitope, and to methods of transferring the partial PLP gene to a host to ameliorate the progression of an immune response to self antigens derived from myelin proteins.

Background of the Invention

The immune system can respond in two ways when exposed to an antigen. A positive response leads to differentiation of T and B cells, antibody production and to immunologic memory. A negative response leads to suppression or inactivation of specific lymphocytes and to tolerance. Tolerance can be defined as the failure of an organism to mount an immune response against a specific antigen. Normally, an organism is tolerant of its own antigens.

Autoimmune diseases are thought to result from an uncontrolled immune response directed against self antigens. In patients with multiple sclerosis (MS), for example, there is evidence that this attack is against the white matter of the central nervous system and more particularly to white matter proteins. Ultimately, the myelin sheath surrounding the axons is destroyed. This can result in paralysis, sensory deficits and visual problems. MS is characterized by a T cell and macrophage infiltrate in the brain. Autoreactive myelin-specific T cells have been isolated from MS patients, although T cells of the same specificity have been detected in normal individuals. J.M. LaSalle et al., J. Immunol. 147:774-780 (1991), J.M. LaSalle et al., J. Exp. Med. 176:177-186 (1992), J. Correale et al., Neurology.45:1370-1378 (1995). Presently, the myelin proteins thought to be the target of an immune response in MS include myelin basic protein (MBP), proteolipid protein (PLP), and myelin-oligodendrocyte glycoprotein (MOG). Individuals who do not mount an autoimmune response to self proteins are thought to have control over these responses and are believed to be "tolerant" of self antigens. The evidence, therefore, that MS is caused by pathogenic T cells is necessarily indirect, but the close resemblance which the characteristics of this disease bear to those

of the murine model, experimental autoimmune encephalomyelitis (EAE), suggest that MS is indeed caused by an aberrant immune response mediated by T cells.

The EAE mouse model for MS, the subject of intense and fruitful study for several years, displays many of the same histopathological and clinical characteristics as the relapsing remitting forms of MS. The T Lymphocyte in Experimental Allergic Encephalomyelitis, Ann. Rev. Immunol. 8:579-621 (1990). EAE can be induced in SJL mice by injection of mouse spinal cord homogenate (MSCH), MBP, PLP, by the injection of synthetic peptides whose sequences correspond to the major encephalitogenic epitopes of myelin basic protein, MBP 84-104, proteolipid protein, PLP 139-151, or by adoptive transfer of activated $CD4^+$ T_{H1}, but not T_{H2} cells specific for encephalitogenic epitopes. The major encephalitogenic epitopes of myelin-derived sequences in EAE, such as MBP, can also activate human T cells of several different haplotypes including HLA-DR2. R. Martin, et al., J. Exp. Med. 173:19-24 (1992). The experimental disease is characterized by a relapsing-remitting course (R-EAE) of neurological dysfunction, perivascular mononuclear infiltration and demyelination. CNS damage is probably mediated by inflammatory cytokines which can activate additional monocytes and macrophages non-specifically. J.E. Blalock, The Immune System. Our Sixth Sense, The Immunologist, 2:8-15 (1994).

Although the initial attack in EAE can be induced by the administration of either T cells specific for MBP or for PLP, close examination of reactivities of T cells in the primary and subsequent relapses demonstrates the presence of T cells which interact with specificities other than the inducing epitopes. This expansion of encephalitogenic epitopes is termed "determinant spreading". S.D. Miller and W.J. Karpus, Immunology

Today 15:356-361 (1994), P.V. Lehman, T. Forsthuber, A. Miller, and E.E. Sercarz, Nature 358-155-157 (1992), H. Jiang, S-I. Zhang and B. Pernis, Science 256:1213-1215 (1992). Antigen specific treatment would therefore, be expected to be more effective when administered early in the course of the disease, before the onset of increasing epitope complexity and eventual non-specific inflammation.

The goal of immunologic therapy is to restore tolerance without suppressing the entire immune system which can lead to complications such as infection, hemorrhage, and cancer. Drugs currently used to treat autoimmune diseases are non-specific immunosuppressive agents, such as anti-inflammatory agents or drugs which can block cell proliferation or depress proinflammatory cytokines. In general, these agents are effective for limited duration and subject to devastating complications.

It is desirable to suppress the immune system in a more specific way to control the response to self-antigens and theoretically "cure" the disease without down-regulating the entire immune system. Several specific immunotherapies have been hypothesized and tested in recent years, many of which are impractical or do not work in humans. For example, high affinity peptides can be synthesized which interact with MHC class II molecules and prevent the binding of encephalitogenic peptides, thereby preventing the activation of pathogenic T cells. A. Franco et al., The Immunologist 2:97-102 (1994). This approach is disadvantageous in that it is difficult to obtain effective concentrations of inhibitor peptides in vivo. G.Y. Ishioka et al., J. Immunol. 152:4310-4319. In an alternate strategy, peptides which are analogs of encephalitogenic sequences have been shown to antagonize the T cell receptors of antigen-specific T cells, rendering them unreactive, although the exact mechanism is at present unknown. S.C. Jameson et al.,

J. Exp. Med 177:1541-1550 (1993), N. Karin et al., J. Exp. Med. 180:2227-2237 (1994), V.K. Kuchroo et al., J. Immunol. 153:3326-3336 (1994). Oral administration of myelin has been tested and found to induce a state of immunological unresponsiveness thought to be mediated by the induction of suppressor T cell or of anergy. H.L. Weiner et al., Annu. Rev. Immunol. 12:809-837 (1994), C.C. Whitacre et al., J. Immunol. 147:2155-2163 (1991), S.J.Khoury et al., J. Exp. Med. 176:1355-1364 (1992). This treatment has been found to be efficacious for some but not all individuals. H.L. Weiner et al., Science 259:1321-1324 (1993). Thus, it is evident that improvements are needed to treat MS and other autoimmune disorders with an effective, immunospecific approach.

Summary of the Invention

The present invention addresses the disadvantages present in the prior art. In general, the invention is based on the discovery that recombinant DNA technology and cell transfer may be employed to restore tolerance to one's own tissues. The present invention provides a means of preparing and constructing a gene, that when expressed and secreted *in vivo*, can provide a means of halting the progression of an autoimmune disease. In further aspects the invention provides a method to construct a gene encoding a portion of a CNS protein, insert the gene sequence into a vector and transfect a cell line. In further aspects, the invention provides a method to construct a gene encoding a portion of a CNS protein, insert the sequence into a retroviral vector, and transduce a producer fibroblast cell line to generate supernatant containing the recombinant retrovirus. Histocompatible fibroblasts are transduced with the recombinant retrovirus encoding a

portion of the CNS protein and are delivered to animals. These fibroblasts continuously secrete a CNS antigen *in vivo* but do not themselves produce viral particles.

In accordance with the present invention, we have used synthetic oligonucleotides to construct a gene encoding a portion of the PLP protein, performed expression of the DNA in combination with various expression vectors, and thereby evaluated expression levels of the gene product *in vitro* and *in vivo*. After transduced histocompatible fibroblasts that secrete the partial PLP protein are transplanted into EAE mice, the disease disappears. The effect is the amelioration of both clinical symptoms and signs and pathological findings.

In a preferred embodiment of the invention, the producer line PA317 is transduced with the PLP retroviral vector to generate supernatant containing the recombinant retrovirus. The producer cell line PA317 was developed by Dr. A. Dusty Miller and has been extensively characterized and approved for human use by the FDA for other clinical trials, such as for genetic diseases and cancer. Miller and Baltimore, Mol. Cell Biol. 6:2895-2902 (1986), W.F. Anderson, Science 256:808-813.

Brief Description of the Drawings

FIGURE 1 is a map of the partial PLP gene showing the sequence of the gene product and restriction sites.

FIGURE 2 is a map of the G1XSvNa vector illustrating restriction sites and functional features. Figure 2b illustrates the entire DNA sequence of G1XSvNa.

FIGURE 3 outlines the method of constructing a G1XSvNa vector containing the PLP gene insert.

FIGURE 4 shows the level of mRNA expressed in transfected and transduced SJL fibroblast cells as detected by reverse transcriptase PCR. Lane 1 is molecular weight standards, Lane 2 is Negative control from mock transfection, Lane 3 is positive control-PLP-gene plasmid, Lane 4 is cDNA from PLP-transfected SJL fibroblasts, Lane 5 is cDNA from PLP transduced SJL fibroblasts.

FIGURE 5 demonstrates the level of PLP protein in the supernatants of transduced fibroblasts as detected by ELISA.

FIGURE 6 demonstrates the level of B-Gal expression in transduced fibroblasts.

FIGURE 7 illustrates the clinical scoring system for chronic EAE.

FIGURE 8 illustrates the histological scoring system for EAE.

FIGURE 9 illustrates the clinical assessment of EAE mice treated with retrovirus transduced fibroblasts.

FIGURE 10a shows the pathologic assessment of brain and spinal cord of SJL mice treated with retrovirus transduced fibroblasts, and 10b is a summary of the pathologic assessment of brain and spinal cord from Days 55-60 through days 90-95.

FIGURE 11 shows the histology of SJL mice with chronic EAE treated with retrovirus transduced fibroblasts.

FIGURE 12 illustrates the results of proliferation assays using EAE mice treated with PLP-expressing fibroblasts.

FIGURE 13 illustrates the results of proliferation assays with and without IL-2 using EAE mice treated with PLP-expressing fibroblasts.

Detailed Description of the Invention

As indicated above, the present invention relates to the use of engineered cells to restore tolerance to self antigens in patients suffering from autoimmune disease. The engineered cells can be any mammalian cell. As used herein, the term "engineered" is

intended to refer to a cell into which one or more recombinant genes, such as a gene encoding an epitope of a self antigen, has been introduced.

A gene is a deoxyribonucleotide sequence coding for an amino acid sequence. Recombinantly introduced genes will either be in the form of a synthetic oligonucleotide, a cDNA gene (i.e. they will not contain introns), a copy of a genomic gene sequence, or a hybrid gene which is a fusion of two or more gene sequences. Optionally the gene may be linked to one or more nucleotide sequence capable of directing expression of the gene product. Sequence elements capable of effecting expression of a gene or gene product include but are not limited to promoters, enhancer elements, transcription termination signals, polyadenylation sites, a Kozak box sequence to ensure efficient translation, and leader sequences. Optionally, the gene sequence can include restriction sites to enable the insertion of additional gene sequences. Preferably, the gene will contain a leader sequence to ensure the gene product is synthesized in the endoplasmic reticulum for later constitutive secretion.

Recombinantly introduced genes carried by the engineered cells can encode one or more epitope, fragment, domain or mini-protein portion of a protein antigen. Examples of suitable proteins from which an epitope, fragment, domain, or mini-protein may be derived include but are not limited to myelin proteins, acetylcholine receptor, TSH receptor, and collagen.

It is believed that protein self-antigens which are the target of an autoimmune response are highly conserved both among and between species. Thus, although the invention will primarily be used to treat humans it can also be used to treat animals. Examples of T cell mediated autoimmune diseases that may be treated using the

invention include but are not limited to multiple sclerosis, myasthenia gravis, systemic lupus erythematosus, psoriasis, juvenile onset diabetes, rheumatoid arthritis, thyroid disease and chronic inflammatory demyelinating polyneuropathy (CIDP).

Expression vectors are generally deoxyribonucleotide molecules engineered for controlled expression of one or more desired genes. The vectors may comprise one or more nucleotide sequences operably linked to a gene to control expression of the desired gene or genes. There are an abundance of expression vectors available and one skilled in the art could easily select an appropriate vector. In addition, standard laboratory manuals on genetic engineering provide recombinant DNA methods and methods for making and using expression vectors. Optionally, the vector may encode a selectable marker, for example, antibiotic resistance.

The gene can be inserted into the mammalian cell using any gene transfer procedure. Examples of such procedures include but are not limited to, RNA viral mediated gene transfer such as retroviral transduction, DNA viral mediated gene transfer, electroporation, calcium phosphate mediated transfection, microinjection or liposome mediated gene transfer. The type of procedure required to achieve an engineered cell that secretes the desired gene product will depend on the nature and properties of the cell. The specific technology for introducing such genes into such cells is generally known and well within the skill of the art.

The examples which follow illustrate the design and construction of a portion of the PLP gene, *in vitro* and *in vivo* expression of the PLP gene product, and the *in vivo* effects of the PLP gene product.

The following examples are presented to illustrate the invention, and are not intended to limit the scope thereof.

EXAMPLE 1

DESIGN AND CONSTRUCTION OF THE PLP GENE

In SJL/J mice, the encephalitogenic epitope of PLP comprises amino acids 139-151. N Takahashi et al., Cell 42:139-148 (1985), K Sakai et al., J. Neuroimmunol. 19:21-32 (1988), D.H. Kono et al., J. Exp. Med. 168:213-227. The vector in the present invention is designed in order that the gene product encoded by it be constitutively secreted from fibroblasts. Since the complete PLP protein is a hydrophobic transmembrane protein (H-J. Diehl, M. Schaich, R-M. Buszinski and W. Stoffel, PNAS U.S.A. 83:9807-9811 (1986)), with the encephalitogenic epitope being extracellular, a plasmid encoding amino acids 101-157 and additional amino acids required for secretion was constructed. This sequence is hydrophilic in character.

1. Oligonucleotide synthesis and construction of the PLP pRc/CMV vector

Oligonucleotides can be synthesized manually, e.g., by the phospho-tri-ester method, as disclosed, for example in R.L. Letsinger, et. al., J. Am. chem. Soc. 98:3655 (1967), the disclosure of which is incorporated by reference. Other methods are well known in the art. See also Matteucci and Caruthers, J. Am. Chem. Soc. 103:3185 (1981), the disclosure of which is incorporated by reference.

Preferably, however, the desired gene sequence can be made by automated synthesis of individual oligonucleotides at .2μM concentrations. For PLP amino acids 101-157, DNA syntheses were performed on a Perkin Elmer/Applied Biosystems Division Model 394 DNA synthesizer using cyanoethyl-protected phosphoramidites. The dimethoxytrityl (DMT) group was not removed from the 5'hydroxyl group to allow for purification. After normal cleavage from the resin using concentrated ammonium hydroxide and deprotection at 55°C for 16 hours, the oligonucleotides were purified using oligonucleotide purification cartridges (OPC) according to the manufacturer's instructions (Applied Biosystems Inc.). Five oligonucleotides of the following sequences were synthesized:

OLG1 5' - CGGCGACTACAAGACCACCATCTGCGGCAAGGGCCTGAGCGC
AACGGTAACAGGGGGCCAGAAGGGGAGGGGTTCCAGAGGCCA
ACATCAAGCTCATTCTCTCGAGC-3',

OLG2 5' - GAGCTTGATGTTGGCCTCTGGAACCCCTCCCCTTCTGGCCCCCT
GTTACCGTTGCGCTCAGGCCCTTGCCGCAGATGGTGGTCTTGTA
GTCGCCGGGCC-3',

OLG3 5' - GGGTGTGTCATTGTTTGGGAAAATGGCTAGGACATCCCGACAA
GTTTGTGGGCATCACCTATGCTAGCCTTAAGTAGGATCCTTGAA
TAGGTA-3',

OLG4 5' - AGCTTACCTATTCAAGGATCCTACTTAAGGCTAGCATAGGTGA
TGCCCA-3',

and

OLG5 5' - CAAACTTGTCGGGATGTCCTAGCCATTTTCCCAAACAATGACA CACCCGCTCGAGAGAAT-3'.

Each purified oligonucleotide was dried under vacuum, washed with 1 ml of sterile double distilled water and then concentrated to dryness under vacuum (Speed vac evaporator; Savant Inc.). 80pM of each oligomer was kinased at 37°C for 1 hour by resuspending in 56.6μl of 1X kinase buffer (Polynucleotide Kinase Buffer; Boehringer Mannheim, Indianapolis, IN) containing 10 units of polynucleotide kinase (Boehringer Mannheim) and 100μM of ATP. The individual oligonucleotides were combined in the presence of 2X SSC (0.03M Sodium Citrate, pH 7.0, and 0.3M NaCl) in a PCR tube with their respective complementary oligomer partners for annealing. Each annealed set measured 200μl in volume. Oligomer OLG1 was annealed with OLG2, and oligomers OLG4 and OLG5 were annealed with OLG3. Annealing was performed in a Perkin-Elmer 9600 Thermocycler, programmed as follows: 1)99.9° for 2 minutes, and 2) 99.9° to 4° in 15 minutes. During the temperature descent to 4°C, when the thermocycler temperature reached 37°C, the solution containing the oligomer duplex OLG1 and OLG2 was combined with the solution containing the oligomers OLG3, OLG4, and OLG5. The descent cycle was then continued until it reached 22°C. Subsequently, 5 units (5μl) of T4 ligase (Boehringer Mannheim, Indianapolis, IN) and 45μl of manufacturer's 10X T4 DNA ligation buffer (Boehinger Mannheim, Indianapolis, IN) was added, and ligation proceeded overnight at 10°C.

The ligated DNA was precipitated with 2 volumes of 100% ethanol and incubated at -70°C for 1 hour. The precipitate was centrifuged for 30 minutes at 17000 x g at 4°C.

The supernatant was discarded and pellet was washed with 1 ml of 70% ethanol and centrifuged for 10 minutes at 17000 x g at 4°C. The DNA pellet was dried under vacuum (Speed vac evaporator; Savant Inc.) and resuspended in 45μl sterile double distilled water.

DNA of the correct molecular weight was isolated by electrophoresis. 5μl of 10x loading buffer (6.25g Ficoll and 0.93g Disodium EDTA/25ml 10% SDS, Orange G, Xylene Cyanole, and Bromophenol Blue) was added to the sample and loaded onto a 14.5 cm x 16cm x 0.15mm urea/acrylamide gel (7M urea/8% acrylamide with 1.1% Bis). TBE (89mM Tris, 89mM Boric acid, and 2mM EDTA pH8.0) was used as both gel and electrophoresis buffer. The sample was electrophoresed at 35mA until the Orange G dye line had migrated within 1 cm of the bottom of the gel. The acrylamide gel was washed twice with water for 5 minutes. After the last wash, the gel was incubated for 3 minutes in a 500 ml solution containing 10ul of 10mg/ml of ethidium bromide, and visualized under a UV-light source. The band corresponding to the ligated DNA was excised and cut into small pieces for electroelution in an IBI electroelutor apparatus (Model UEA: International Biotechnologies Inc., New Haven, CT).

For electroelution, the salt trap of the apparatus was filled with 125μl of 7M sodium acetate/bromophenol blue dye solution. The buffer chamber was filled with 1/2X TBE. The sample was electroeluted for 1 hour at 85V. After removing the eluted DNA, the sample well was washed with 1/2X TBE and combined with the initial eluate. The eluted DNA was then precipitated overnight at -70°C with 2 volumes of 100% ethanol. The precipitate was pelleted, washed as previously described, and resuspended in 15ul of sterile double distilled water.

Preceding the ligation of the eluted partial PLP gene to the pRc/CMV vector (Invitrogen, San Diego, CA), the pRc/CMV vector construct was cut with the restriction endonucleases Apa I and Hind III according to the Manufacturer's instructions (Boehringer Mannheim, Indianapolis, IN). The resuspended PLP gene construct was then added to a 5μl mixture containing 0.3μg of pRc/CMV cut vector (2μl), 1 unit T4 ligase (1μl) (Boehringer Mannheim, Indianapolis, IN), and 2μl of Manufacturer's 10X T4 DNA ligation buffer (Boehringer Mannheim, Indianapolis, IN). The ligated vector was then transformed into the competent cell line AG1.

Transformation proceeded by combining the ligation mixture with the AG1 cells and incubating it on ice for 20 minutes. The cell/vector mixture was then incubated at 42° for 2 minutes and plated overnight onto a Luria Broth agar (LB; Bio101, Vista, CA) plate, supplemented with 80 mg/ml of ampicillin (Sigma, St. Louis MO). Colonies were screened for the correct sequence vector by first isolating the plasmid DNA and then sequencing the DNA.

To isolate the plasmid, a commercially available plasmid purification kit, Wizard Minipreps (Promega, Madison, WI) was used. Colonies were picked from the LB/Amp plates and grown for 3.5 hours in 5 ml of LB medium (BIO 101, Vista, CA) supplemented with 80mg/ml of ampicillin (Sigma, St. Louis, MO). 3 ml of the medium was centrifuged at 17000 x g at room temperature, for 1 minute to pellet the cells. Isolation of the plasmid proceeded according to the Manufacturer's instructions. 1μg of the isolated DNA was used for sequencing.

The oligonucleotide sequence can be checked by methods well known in the art, such as that described by Sanger et. al., PNAS U.S.A. 70:1209 (1973) or by the Maxam-

Gilbert method, Meth. Enzymology, 65:499 (1977), the disclosures of both of which are incorporated herein by reference. Preferably, the plasmid can be sequenced using an automated DNA sequencer. For the PLP pRc/CMV construct, the plasmid was sequenced using automated fluorescent DNA sequencing procedures (Perkin Elmer/Applied Biosystems Inc, Foster City, CA) using the following primers: GATTTAGGTGACACTATAG and TAATACGACTCACTATAGGG. These primers primed off the vector, which flanked the Kozak and "stop" site of the total construct. Figure 1 shows a map of the partial PLP gene showing the sequence of the gene product and restriction sites. At the 5' end of the construct we had previously inserted a hydrophobic leader sequence from the MHC class I L^d gene to enable the gene product to be synthesized in the endoplasmic reticulum (ER) for later constitutive secretion. Linsk et al. J.Exp. Med. 164:794-813 (1996). In addition, a lysine codon at the 3' end was added to ensure that the protein could not be retained in membrane. A Kozak box was included in the construct to ensure efficient translation. Restriction sites Afl II and BamHI were included in the construct to allow for insertion of further epitopes.

EXAMPLE 2

IN VITRO EXPRESSION OF THE PLP PROTEIN

The following experiments were performed in order to demonstrate that the PLP vector encodes a protein which is constitutively secreted. Specifically, the mRNA levels of PLP were evaluated in SJL fibroblast cells transfected with the pRc/CMV-PLP vector, and mRNA and protein levels of PLP were evaluated in SJL fibroblast cells transfected with the pG1PLPSvNa vector.

1. Establishment of Fibroblast Cultures

Syngeneic fibroblasts (derived from SJL mice) were obtained from Dr. G. Dveskler (Uniformed Services University, Bethesda, MD) and expanded at 37° incubation using DMEM growth medium, supplemented with 5% glutamine and 10% FCS. The cells were harvested and frozen at 1 x 10^7 cells per vial, and aliquots were quality control tested for mycoplasma, sterility and viability.

2. Retroviral Constructs

A recombinant retroviral vector in which exogenous genes are inserted into a retroviral vector was constructed. The cloning strategy was to construct a pG1XSvNa vector (W. French Anderson, University of Southern California) containing the PLP insert from pRc/CMV-PLP. The pG1XSvNa vector, like most retroviral vectors used in preclinical and clinical trials, is derived from the Moloney murine leukemia retrovirus (Mo-MLV). Rosenberg et al., N. Eng. J. Med. 323:570-578 (1990), Culver et al., Science 256:1550-1552 (1992). The G1XSvNa vector is a 5865 bp vector whose map, functional features and complete DNA sequence are shown in Figures 2a and 2b. Figure 3 illustrates the procedure for constructing the pG1PLPSvNa vector. Essentially, the pRc/CMV-PLP vector was digested with BstEII/HindIII and PLP encoding fragment was isolated by gel electrophoresis. After electroelution, HindIII/NotI adapters (Stratagene, La Jolla, CA) were ligated into the HindIII site of the eluted fragment. A NotI digestion was performed to generate NotI ends. A NotI digest was performed on pG1XSvNa and the 5865 bp fragment was isolated, electroeluted, and a CIAP (Calf intestine alkaline phosphatase treatment) was performed on the fragment ends. The NotI site of the insert

was ligated into the NotI site of the vector. BstEII ends of the insert and NotI site of the vector were Klenowed. A blunt end ligation is performed to close the vector. HB101 cells were transformed with ligation mix and restriction analysis was performed to determine which vectors contain insert and the insert orientation. The recombinant retroviruses are non-replicating and incapable of producing infectious virus.

3. Retroviral vector supernatant

To prepare supernatant containing PLP-recombinant retrovirus, the PLP-transduced retroviral packaging cell line PA317 was grown in 4 ml of appropriate culture medium in a T25 flask (Corning, Cambridge, MA). Retroviral vector supernatant is produced by harvesting the cell culture medium when cells were 80-90% confluent, and stored in 1 ml aliquots at -70C°.

The following tests were performed on the PLP cell line and/or the vector supernatants:

(1) The viral titer is determined using 3T3 cells. Viral preparations with titers greater than 5 X 10^4 colony forming units/ml are used.

(2) Sterility of the producer cell line and the supernatant is assured by testing for aerobic and anaerobic bacteria, fungus and mycoplasma.

The PLP-vector preparations from PA317 can be extensively tested to assure that no detectable replication competent virus is present. This is particularly relevant to the embodiment of the invention wherein the invention is used to treat humans. Tests on both the viral supernatant and on the transduced fibroblasts can be performed to

determine if there is replication competent virus present. The following tests can be performed on the producer cell line and/or the viral supernatant:

(1) The viral titer is determined using 3T3 cells. Viral preparations with titers greater than 5 X 10^4 colony forming units/ml are used.

(2) Southern blots are run on the producer cell line to detect the partial PLP gene.

(3) PLP production by the producer cell line is measured and should be significantly above baseline control values, as determined by ELISA assay.

(4) Sterility of the producer cell line and the supernatant is assured by testing for aerobic and anaerobic bacteria, fungus and mycoplasma.

(5) Viral testing is performed including: MAP test, LCM virus, thymic agent, S + L-assay for ecotropic virus, S + L assay for xenotropic virus, S + L-assay for amphotropic virus and 3T3 amplification.

(6) Electron microscopy is performed to assure the absence of adventitious agents.

Following the introduction of the gene into fibroblasts, the following tests are performed on the fibroblasts prior to administration to patients.

(1) Cell viability is greater than 70% as tested by trypan blue dye exclusion.

(2) Cytologic analysis is performed on over 200 cells prior to infusion to assure that tumor cells are absent.

(3) Sterility is assured by testing for aerobic and anaerobic bacteria, fungus and mycoplasma.

(4) S + L-assay including 3T3 amplification must be negative.

(5) PCR assay for the absence of 4070A envelope gene must be negative.

(6) Reverse transcriptase assay must be negative.

(7) Southern blots run on the transduced fibroblasts to assure that intact provirus is present.

(8) PLP protein assay to assure the production of PLP protein.

4. Transfection of fibroblasts

Prior to the transfection of the SJL fibroblasts, highly purified PLP-pRc/CMV vector was isolated from the transformed AG1 cells. Large scale purification was performed by using a commercially available kit and CsCl gradient banding. Initial purification was accomplished using a Wizard Megaprep Kit (Promega, Madison, WI). A 1000ml culture of transformed AG1 cells, grown overnight in LB/Amp at 37°C, was pelleted and the plasmid DNA isolated according to the Manufacturer's instructions. The isolated DNA, which was suspended in 3 ml of TE buffer (10mM Tris-HCl, pH 7.4, and 1mM disodium EDTA, pH, 8.0) was further processed by CsCl gradient banding. A modified CsCl banding of the DNA was performed based on procedures found in "Current Protocols in Molecular Biology, Vol 1" (Greene Publishing Associates and Wiley-Interscience).

After the DNA band was extracted from the ultracentrifuge tubes, ethidium bromide was removed from the sample by washing it with 3 volumes of SSC saturated isopropanol. The wash was repeated until the aqueous layer appeared clear. CsCl was removed by precipitation. 2 volumes of 0.2M NaCl/TE and 2 volumes of 100% ethanol (relative to the combined total volume of DNA solution and 0.2M NaCl/TE) were added

to the sample, mixed and placed on ice for 10 minutes. The precipitated DNA was pelleted by centrifugation at 10000 x g for 10 minutes at 4°C. The pellet was washed with cold 70% ethanol, recentrifuged at 10000 x g for 10 minutes at 4°C, and dried under vacuum (Speed vac evaporator; Savant Inc.). The purified DNA was resuspended with double-distilled sterile water and utilized in the transfection process.

Test SJL fibroblasts were transfected using LipofectAMINE Reagent (Life Technologies Inc./Gibco BRL) according to the manufacturer's instructions. Control SJL fibroblasts underwent the same procedure without the presence of a DNA construct. 3μg of CsCl purified PLP-pRc/CMV plasmid and 25μl of Lipofectamine were used for transfection. Approximately 3 X 10^5 SJL cells, seeded overnight into 25cm ^{2}culture flasks (Corning Costar Corp., Cambridge, MA.) and grown at 37° with 5% CO_2 in 5ml of DMEM culture medium (Dulbecco's Modified Eagle's Medium (Irvine Scientific, Santa Ana, CA), supplemented with 5% glutamine, 10% Fetal Calf Serum, 25 Units/ml of penicillin G sodium, and 25μg/ml of streptomycin sulfate, were washed with 3ml serum free HL-1 medium (Hycor Biomedical Inc., Irvine, CA). After the DNA/lipofectamine complexes were incubated with cells for 6 hours at 37° with 5% Co_2. 1 ml of DMEM was added to the flasks. The flasks were incubated overnight at 37° with 5% CO_2. The medium was replaced with 5ml of fresh DMEM the next morning. 36 hours after the end of the transfection period, the medium was replaced with 5ml of DMEM containing 900μg of G418 (Life Technologies Inc./Gibco BRL)/ml of medium. The test cells were grown in the presence of 900μg of G418 of medium until all the control cells had died; and no more cell death could be observed in the test sample flask.

The G418 concentration was then reduced to 600μg/ml of culture medium for duration of cell culturing procedures.

5. Transduction of Fibroblasts

Retroviral constructs containing a neo-selectable marker together with either the PLP gene or the Lac-z gene were used to transduce fibroblasts. Transduction with the retrovirus was performed on healthy cells (90% viable, as determined by trypan blue staining). 2 X 10^6 cells were plated in 0.5 ml DMEM-10 media (DMEM media supplemented with 10% fetal calf serum, 2 mM L-glutamine, 50 U/ml penicillin G, 50 mg/ml streptomycin in one well of a 24-well plate (Falcon, Franklin Lakes, NJ). Cells were placed in the incubator and allowed to settle (37°, 5% CO_2). After cells had settled, 1 ml of retroviral supernatant and polybrene (Sigma, St. Louis, MO) (final concentration 10μg/ml) was added to the well. Cells were incubated as above for 2.5 hours without shaking. After 2.5 hours, cells were transferred to a T25 flask and DMEM-10 media was added to a total volume of 8 ml. Selection media (culture media comprising DMEM-10 supplemented with 900 μg/ml G418 (Gibco, Grand Island, NY) was added on the third day after transduction. The G418 concentration was then reduced to 600μg/ml of culture medium for the duration of cell culturing procedures.

6. mRNA expression analysis

mRNA isolation was performed using aseptic techniques, RNAse free supplies, and DEPC (Diethylpyrocarbonate) treated solutions. 4 X 10^6 experimental and control SJL cells were washed twice with cold Phosphate-buffered saline, resuspended in 200μl

cell lysis mix (10mM TRIS pH 7.5, 0.15M NaCl, 1.5mM $MgCl_2$, 0.65% NP 40), vortexed, and centrifuged at 17000 x g at 4° for 5 minutes. The supernatant was transferred to a tube containing 200μl of urea mix (7M urea, 1% SDS, 0.35M NaCl, 10mM EDTA, and 10mM Tris-HCL, pH 7.5) and 400μl of phenol:chloroform:isoamyl alcohol (25:24:1). The solution was vortexed and centrifuged for 1 minute at 17000 x g. This procedure was repeated twice using the aqueous layer and then transferred to a tube containing 400μl of phenol and washed as before. The aqueous layer was transferred again to another tube, and precipitated with 1ml of 100% ethanol overnight at -20°C. The precipitated RNA was washed with 1ml 70% ethanol. After the ethanol was discarded, the pellet was dried under vacuum. 1μg of the RNA was used for RT-PCR analysis.

RT-PCR was performed using a commercially available kit, GeneAmp RNA PCR Kit (Perkin Elmer/ABI) according to the Manufacturer's instructions. The following primers were used to amplify the cDNA: 5'-GCGACTACAAGACCACCATCT-3' and 5'-TAAGGCTAGCATAGGTGATG-3'. The PCR products were electrophoresed on a 1.5% agarose (SeaKem GTG; FMC)/TAE gel with 1μl of 10mg/ml of ethidium bromide/ml of agarose solution. The gel was electrophoresed using TAE buffer at a constant 40mA. Electrophoresis was continued until the molecular weight marker bands had separated adequately enough, to verify the PCR products' approximate molecular size. The DNA band of interest was then excised and gel purified, using the commercially available MERmaid Kit (Bio 101, Vista, CA), according to the Manufacturer's instructions. The purified DNA was then sequenced by automated Fluorescent DNA sequencing procedures (Perkin Elmer/ABI, Foster City, CA).

Figure 4 is an agarose gel showing PLP-specific RT-PCR products. The data illustrates that mRNA is present in both PLP-transduced and PLP-transfected cells. The correlation between mRNA and secreted protein remains to be determined since peptide concentration does not necessarily correspond to the level of mRNA.

7. Protein Expression Analysis

The in vitro qualitative expression of the proteins encoded by the PLP gene was detected immunologically by ELISA. Undiluted supernatants from cultures of fibroblasts transduced with the PLP gene were tested. Wells of 96 microtiter plate were coated with the supernatants. Primary anti-PLP-antibody 4E10 139-151, from Dr. M. Lees (Harvard), is specific for PLP 139-151 and was added to wells as undiluted hybridoma supernatant followed by horseradish peroxidase (HRP)-conjugated goat anti-mouse secondary antibody in a concentration of 1:500. The plate was developed and analyzed at 490 nm on a microplate reader. Figure 5 illustrates the results of ELISA assays on transduced fibroblast supernatants. Samples 1 and 2 were PLP (amino acids 139-151) and HIV gp120 peptides used at a concentration of 5ug/ml. This experiment illustrates that the transduced PLP-transduced fibroblasts do produce and secrete the partial PLP protein.

EXAMPLE 3

IN VIVO EFFECTS OF THE PLP PROTEIN

Critical to the success of this invention in the embodiment of this example is the ability to deliver genetically manipulated fibroblasts to patients so that the cells survive

in sufficient numbers and for long periods of time, in order that continuous secreted antigen may be provided to the patient.

To assess the fate of transplanted transduced fibroblasts, SJL fibroblasts transduced with retrovirus encoding B-galactosidase were injected subcutaneously between the shoulders of SJL mice. All mice were female mice of the SJL strain between 6-8 weeks old and were obtained from Jackson Labs. Animals were housed and maintained according to NIH guidelines (National Research Council, 1986). These fibroblasts survived in large numbers after 60 days. Fibroblasts injected into the footpad or intramuscularly could not be detected at eight days.

1. In *Vivo* fate B-gal transduced cells

The activity of the B-Galactosidase marker was evaluated using two groups of eight normal mice. Two mice were injected subcutaneously on the back, two mice were injected intramuscularly and two mice were injected in the footpad with Lac-Z transduced cells. One animal was injected with fibroblasts transduced with neo-marker only, and the last mouse was injected with untransduced fibroblasts. After harvesting and washing, the different cell lineages were suspended in a concentration of 10^7 cells in .2 ml of Hank's PBS and slowly injected using a 25 gauge needle at different sites. Animals were sacrificed at 10 and 15 days post treatment and injection sites were submitted to histochemical study. Pieces of tissue were fixed in 4% paraformaldehyde for one hour, washed in PBS three times and then kept in 8.4% acrylamide solution overnight. The next morning tissues were embedded in acrylamide which after hardening were cut and frozen. The frozen sections were done in 10um by cryostat and stained with

1 ml of 5-Bromo-4-chloro-3-indolyl-B-d-galactopyranoside (X-Gal) in PBS. The X-Gal was dissolved in DMSO at 40mg/ml and then added to the reaction mixture. Incubation was for 14-18 h at 37°. Figure 6 illustrates B-Gal expression in transduced fibroblasts 60 days in vivo. There was no evidence of an inflammatory response, suggesting that the retrovirus used to transduce syngeneic fibroblasts, does not evoke an immune response or rejection process.

2. Effect of PLP in normal SJL mice

Another important aspect of this invention in the embodiment of this example is determining whether transduced fibroblasts secreting PLP actually produce EAE in normal animals. To test this, 10^7 PLP-secreting SJL fibroblasts were injected into 12 normal SJL mice. Six animals had fibroblasts placed subcutaneously and six animals had fibroblasts injected intraperitoneally. Animals were sacrificed at day 16 and showed no evidence of inflammatory disease or EAE. Figure 7 illustrates the clinical scoring system for chronic EAE. Y-A Lu et al., Mol. Immunol.. 28:623-630 (1991), J. Williamson et al., J. Neuroimmunol. 32:199-207 (1991). In the EAE model for multiple sclerosis, using spinal cord homogenates plus adjuvant, inflammation in the CNS can be seen by day 14. In this study, normal animals injected with PLP-secreting SJL fibroblasts did not show any signs of clinical disease even at day 60. In addition, the animals did not show any histologic evidence of inflammation in the CNS at day 60. Figure 8 illustrates the histological scoring system for EAE. J. Governman et al., Cell 72:551-560 (1993).

3. Clinical and histological assessment of acute EAE mice treated with retrovirus transduced fibroblasts.

Six week SJL mice were infected with mouse spinal cord homogenate (MSCH) in complete Freund's Adjuvant (CFA) and with MSCH in incomplete Freund's Adjuvant IFA, seven days later. J. Immunol. 144:909-915 (1990). The initial EAE attack was observed on days 14-18, with full recovery by 21. Ninety-five percent of animals showed clinical evidence of an acute attack and these were given either 10^7 PLP secreting SJL fibroblasts or control fibroblasts on day 21. Animals not showing clinical disease were eliminated from the experiment. Figure 9 illustrates the clinical assessment of EAE mice treated with retrovirus transduced fibroblasts. Animals receiving the PLP secreting fibroblasts had a marked reduction of clinical signs and had dramatic reduction in inflammatory cells, particularly in the brain. Figure 10a illustrates the pathologic assessment of brain and spinal cord of SJL mice treated with retrovirus transduced fibroblasts. Figure 10b is a summary of the pathologic assessment of brain and spinal cord from days 55-60 and 90-95. Histological assessment of EAE Grades in Brain and Spinal Cord were performed following the preparation of hematoxylin and eosin stained sections.

4. Clinical and histological assessment of chronic EAE mice treated with retrovirus transduced fibroblasts.

150 mice were inoculated with MSCH in CFA. A second immunization was given 7 days later. A.M. Brown and D.E. McFarlin, Laboratory Invest. 45:278-284 (1981). On day +14 to 16, 113 animals developed clinical disease lasting 3-4 days. These positive animals were separated for subsequent experiments and had their first relapse on

day +55 to 60, with 100 animals becoming sick. These were again separated and on day +137, 67 had a relapse. Eight days after relapse, animals were each transplanted with 10^7 fibroblasts and then sacrificed 18 to 23 days later. Four different types of fibroblasts were used, those transduced with retrovirus encoding PLP, encoding B-galactosidase and encoding neo-selectable marker as well as untransduced cells. Figure 11 shows the histology of SJL mice with chronic EAE treated with retrovirus transduced fibroblasts. There were no animals receiving PLP secreting fibroblasts with 2+ to 3+ inflammation.

5. Peripheral immune status of treated mice v. control EAE mice.

Spleen cells from our EAE control mice and from four EAE mice which had been treated with fibroblasts expressing the PLP protein were used in proliferation assays, in which they were incubated with 40μM PLP peptide 139-151 or 40μM HIV gp120 peptide 308-322 for 4 days and then pulsed with ^{3}H-thymidine for 24 hours.

Briefly, animals were sacrificed by CO_2 asphyxiation. Spleen cells were dispersed to single cell suspensions in RPMI 1640 by passing through a size 60 mesh, and washed once before being cultured (8 x 10^5 per well) in 0.2 ml of HL-1 medium (Hycor Biomedical, Irvine, CA), supplemented with 2mM glutamine, 100U/ml penicillin, 100μg streptomycin either alone or with 40μM of peptide in 96-well tissue culture plates for 4 days at 37°C with 5% CO_2. PLP peptide 140-151 and MBP peptide 89-101 were used for antigen-specific proliferation while HIV gp120 peptide 308-322 was used as negative control. Where indicated, some wells also contained 10U/ml of recombinant mouse IL-2 (Boehringer Mannheim, Indianapolis, IN). During the last 18-24 h of culture, each well was pulsed with 1μCi of ^{3}H-thymidine (ICN, Irvine, CA), harvested onto 'Xtal Scint'

glass fiber filters (Beckman, Fullerton, CA) and counted using a Beckman LS6000 Scintillation counter. Thymidine incorporation values (experimental counts per minute - background counts per minute) were calculated and represent means of triplicate cultures ± standard deviation.

The results are shown in Figure 12 and suggest that PLP specific proliferative responses are reduced significantly in EAE mice which have received PLP expressing fibroblasts.

Figure 13 illustrates the same experiment as in Figure 12 but with the addition of mouse IL-2 (10U/ml) for 5 days. These results illustrate that the mechanism by which the PLP specific proliferative responses are reduced significantly may suggest the possibility of deletion of T cells rather than anergy because these lymphocytes do not respond to IL-2.

Although the mechanism by which the present invention acts to restore tolerance in individuals suffering from T-cell mediated autoimmune disease is not entirely understood, the benefits of the treatment are clearly advantageous over alternative treatments. The method is a genetic approach to immunospecifically silence pathogenic T-cell responses and does not down-regulate the entire immune system. In the case where an individual with a T-cell mediated autoimmune disease exhibits pathogenic T-cells of multiple specificities, the invention may easily be adapted to target those specificities. For example, DNA encoding multiple self-antigenic epitopes may be introduced into the patient's cells. The invention is also advantageous in that the reagents can easily be made or obtained in sufficient quantity to carry out the invention.

The present invention is not to be limited in scope by the exemplified embodiments disclosed herein which are intended as illustrations of single aspects of the invention, and clones, DNA or amino acid sequences which are functionally equivalent are within the scope of the invention. Various modifications of the invention, in addition to those shown and described herein, will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

Various publications are cited herein that are hereby incorporated by reference in their entireties.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Weiner, Leslie P.
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(ii) TITLE OF INVENTION: Construction and Use of Genes Encoding Pathogenic Epitopes For Treatment of Autoimmune Disease

(iii) NUMBER OF SEQUENCES: 2

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(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Diskette, 3.50 inch

(B) COMPUTER: IBM

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(viii) ATTORNEY/AGENT INFORMATION:

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(2) INFORMATION FOR SEQ ID NO: 1

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 317 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1

GATGGTGACC	GGAGATCTGC	CGCCACCATG
GGGGCGATGG	CTCCGCGCAC	GCTGCTCCTG
CTGCTGGCGG	CCGCCCTGGC	CCCGACTCAG
ACCCGCGCGG	GGCCCGGCGA	CTACAAGACC
ACCATCTGCG	GCAAGGGCCT	GAGCGCAACG
GTAACAGGGG	GCCAGAAGGG	GAGGGGTTCC
AGAGGCCAAC	ATCAAGCTCA	TTCTCTCGAG
CGGGTGTGTC	ATTGTTTGGG	AAAATGGCTA
GGACATCCCG	ACAAGTTTG	TGGGCATCAC
CTATGCTAGC	CTTAAGTAGG	ATCCTTGAAT
AGGTAAGTTG	CTAGCCC	

(2) INFORMATION FOR SEQ ID NO: 2

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5865 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Double

(D) TOPOLOGY: Circular

(ii) MOLECULE TYPE: Vector DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2

1	TTTGAAAGAC	CCCACCCGTA	GGTGGCAAGC	TAGCTTAAGT	AACGCCACTT	TGCAAGGCAT
61	GGAAAAATAC	ATAACTGAGA	ATAGAAAAGT	TCAGATCAAG	GTCAGGAACA	AAGAAACAGC
121	TGAATACCAA	ACAGGATATC	TGTGGTAAGC	GGTTCCTGCC	CCGGCTCAGG	GCCAAGAACA
181	GATGAGACAG	CTGAGTGATG	GGCCAAACAG	GATATCTGTG	GTAAGCAGTT	CCTGCCCCGG
241	CTCGGGGCCA	AGAACAGATG	GTCCCCAGAT	GCGGTCCAGC	CCTCAGCAGT	TTCTAGTGAA
301	TCATCAGATG	TTTCCAGGGT	GCCCCAAGGA	CCTGAAAATG	ACCCTGTACC	TTATTTGAAC
361	TAACCAATCA	GTTCGCTTCT	CGCTTCTGTT	CGCGCGCTTC	CGCTCTCCGA	GCTCAATAAA
421	AGAGCCCACA	ACCCCTCACT	CGGCGCGCCA	GTCTTCCGAT	AGACTGCGTC	GCCCGGGTAC
481	CCGTATTCCC	AATAAAGCCT	CTTGCTGTTT	GCATCCGAAT	CGTGGTCTCG	CTGTTCCTTG
541	GGAGGGTCTC	CTCTGAGTGA	TTGACTACCC	ACGACGGGGG	TCTTTCATTT	GGGGGCTCGT
601	CCGGGATTTG	GAGACCCCTG	CCCAGGGACC	ACCGACCCAC	CACCGGGAGG	TAAGCTGGCC
661	AGCAACTTAT	CTGTGTCTGT	CCGATTGTCT	AGTGTCTATG	TTTGATGTTA	TGCGCCTGCG
721	TCTGTACTAG	TTAGCTAACT	AGCTCTGTAT	CTGGCGGACC	CGTGGTGGAA	CTGACGAGTT
781	CTGAACACCC	GGCCGCAACC	CTGGGAGACG	TCCCAGGGAC	TTTGGGGGCC	GTTTTTGTGG
841	CCCGACCTGA	GGAAGGGAGT	CGATGTGGAA	TCCGACCCCG	TCAGGATATG	TGGTTCTGGT
901	AGGAGACGAG	AACCTAAAAC	AGTTCCCGCC	TCCGTCTGAA	TTTTTGCTTT	CGGTTTGGAA
961	CCGAAGCCGC	GCGTCTTGTC	TGCTGCAGCG	CTGCAGCATC	GTTCTGTGTT	GTCTCTGTCT
1021	GACTGTGTTT	CTGTATTTGT	CTGAAAATTA	GGGCCAGACT	GTTACCACTC	CCTTAAGTTT
1081	GACCTTAGGT	CACTGGAAAG	ATGTCGAGCG	GATCGCTCAC	AACCAGTCGG	TAGATGTCAA
1141	GAAGAGACGT	TGGGTTACCT	TCTGCTCTGC	AGAATGGCCA	ACCTTTAACG	TCGGATGGCC

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1201  GCGAGACGGC  ACCTTTAACC  GAGACCTCAT  CACCCAGGTT  AAGATCAAGG  TCTTTTCACC
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1321  TGACCCCCCT  CCCTGGGTCA  AGCCCTTTGT  ACACCCTAAG  CCTCCGCCTC  CTCTTCCTCC
1381  ATCCGCCCCG  TCTCTCCCCC  TTGAACCTCC  TCGTTCGACC  CCGCCTCGAT  CCTCCCTTTA
1441  TCCAGCCCTC  ACTCCTTCTC  TAGGCGCCGG  AATTCGCGGC  CGCTACGTAG  TCGACTCGCT
1501  GTGGAATGTG  TGTCAGTTAG  GGTGTGGAAA  GTCCCCAGGC  TCCCCAGCAG  GCAGAAGTAT
1561  GCAAAGCATG  CATCTCAATT  AGTCAGCAAC  CAGGTGTGGA  AAGTCCCCAG  GCTCCCCAGC
1621  AGGCAGAAGT  ATGCAAAGCA  TGCATCTCAA  TTAGTCAGCA  ACCATAGTCC  CGCCCCTAAC
1681  TCCGCCCATC  CCGCCCCTAA  CTCCGCCCAG  TTCCGCCCAT  TCTCCGCCCC  ATGGCTGACT
1741  AATTTTTTTT  ATTTATGCAG  AGGCCGAGGC  CGCCTCGGCC  TCTGAGCTAT  TCCAGAGTA
1801  GTGAGGAGGC  TTTTTTGGAG  GCCTAGGCTT  TTGCAAAAAG  CTCGAAGATC  AATTCCGATC
1861  TGATCAAGAG  ACAGGATGAG  GATCGTTTCG  CATGATTGAA  CAAGATGGAT  TGCACGCAGG
1921  TTCTCCGGCC  GCTTGGGTGG  AGAGGCTATT  CGGCTATGAC  TGGGCACAAC  AGACAATCGG
1981  CTGCTCTGAT  GCCGCCGTGT  TCCGGCTGTC  AGCGCAGGGG  CGCCCGGTTC  TTTTTGTCAA
2041  GACCGACCTG  TCCGGTGCCC  TGAATGAACT  GCAGGACGAG  GCAGCGCGGC  TATCGTGGCT
2101  GGCCACGACG  GGCGTTCCTT  GCGCAGCTGT  GCTCGACGTT  GTCACTGAAG  CGGGAAGGGA
2161  CTGGCTGCTA  TTGGGCGAAG  TGCCGGGGCA  GGATCTCCTG  TCATCTCACC  TTGCTCCTGC
2221  CGAGAAAGTA  TCCATCATGG  CTGATGCAAT  GCGGCGGCTG  CATACGCTTG  ATCCGGCTAC
2281  CTGCCCATTC  GACCACCAAG  CGAAACATCG  CATCGAGCGA  GCACGTACTC  GGATGGAAGC
2341  CGGTCTTGTC  GATCAGGATG  ATCTGGACGA  AGAGCATCAG  GGGCTCGCGC  CAGCCGAACT
2401  GTTCGCCAGG  CTCAAGGCGC  GCATGCCCGA  CGGCGAGGAT  CTCGTCGTGA  CCCATGGCGA
2461  TGCCTGCTTG  CCGAATATCA  TGGTGGAAAA  TGGCCGCTTT  TCTGGATTCA  TCGACTGTGG
2521  CCGGCTGGGT  GTGGCGGACC  GCTATCAGGA  CATAGCGTTG  GCTACCCGTG  ATATTGCTGA
2581  AGAGCTTGGC  GGCGAATGGG  CTGACCGCTT  CCTCGTGCTT  TACGGTATCG  CCGCTCCCGA
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2701  TTCGTCGAGA  AGCTTGGGCC  CATCGATAAA  ATAAAAGATT  TTATTTAGTC  TCCAGAAAAA
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2941  AGGGCCAAGA  ACAGATGGAA  CAGCTGAATA  TGGGCCAAAC  AGGATATCTG  TGGTAAGCAG
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3061  GTTTCTAGAG  AACCATCAGA  TGTTTCCAGG  GTGCCCCAAG  GACCTGAAAT  GACCCTGTGC
3121  CTTATTTGAA  CTAACCAATC  AGTTCGCTTC  TCGCTTCTGT  TCGCGCGCTT  CTGCTCCCCG
3181  AGCTCAATAA  AAGAGCCCAC  AACCCCTCAC  TCGGGGCGCC  AGTCCTCCGA  TTGACTGAGT
3241  CGCCCGGGTA  CCCGTGTATC  CAATAAACCC  TCTTGCAGTT  GCATCCGACT  TGTGGTCTCG
3301  CTGTTCCTTG  GGAGGGTCTC  CTCTGAGTGA  TTGACTACCC  GTCAGCGGGG  GTCTTTCATT
3361  TGGGGGCTCG  TCCGGGATCG  GGAGACCCCT  GCCCAGGGAC  CACCGACCCA  CCACCGGGAG
3421  GTAAGCTGGC  TGCCTCGCGC  GTTTCGGTGA  TGACGGTGAA  AACCTCTGAC  ACATGCAGCT
3481  CCCGGAGACG  GTCACAGCTT  GTCTGTAAGC  GGATGCCGGG  AGCAGACAAG  CCCGTCAGGG
3541  CGCGTCAGCG  GGTGTTGGCG  GGTGTCGGGG  CGCAGCCATG  ACCCAGTCAC  GTAGCGATAG
3601  CGGAGTGTAT  ACTGGCTTAA  CTATGCGGCA  TCAGAGCAGA  TTGTACTGAG  AGTGCACCAT
3661  ATGCGGTGTG  AAATACCGCA  CAGATGCGTA  AGGAGAAAAT  ACCGCATCAG  GCGCTCTTCC
3721  GCTTCCTCGC  TCACTGACTC  GCTGCGCTCG  GTCGTTCGGC  TGCGGCGAGC  GGTATCAGCT
3781  CACTCAAAGG  CGGTAATACG  GTTATCCACA  GAATCAGGGG  ATAACGCAGG  AAAGAACATG
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3841  TGAGCAAAAG GCCAGCAAAA GGCCAGGAAC CGTAAAAAGG CCGCGTTGCT GGCGTTTTTC
3901  CATAGGCTCC GCCCCCCTGA CGAGCATCAC AAAAATCGAC GCTCAAGTCA GAGGTGGCGA
3961  AACCCGACAG GACTATAAAG ATACCAGGCG TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT
4021  CCTGTTCCGA CCCTGCCGCT TACCGGATAC CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG
4081  GCGCTTTCTC AATGCTCACG CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG
4141  CTGGGCTGTG TGCACGAACC CCCCGTTCAG CCCGACCGCT GCGCCTTATC CGGTAACTAT
4201  CGTCTTGAGT CCAACCCGGT AAGACACGAC TTATCGCCAC TGGCAGCAGC CACTGGTAAC
4261  AGGATTAGCA GAGCGAGGTA TGTAGGCGGT GCTACAGAGT TCTTGAAGTG GTGGCCTAAC
4321  TACGGCTACA CTAGAAGGAC AGTATTTGGT ATCTGCGCTC TGCTGAAGCC AGTTACCTTC
4381  GGAAAAAGAG TTGGTAGCTC TTGATCCGGC AAACAAACCA CCGCTGGTAG CGGTGGTTTT
4441  TTTGTTTGCA AGCAGCAGAT TACGCGCAGA AAAAAAGGAT CTCAAGAAGA TCCTTTGATC
4501  TTTTCTACGG GGTCTGACGC TCAGTGGAAC GAAAACTCAC GTTAAGGGAT TTTGGTCATG
4561  AGATTATCAA AAAGGATCTT CACCTAGATC CTTTTAAATT AAAAATGAAG TTTTAAATCA
4621  ATCTAAAGTA TATATGAGTA AACTTGGTCT GACAGTTACC AATGCTTAAT CAGTGAGGCA
4681  CCTATCTCAG CGATCTGTCT ATTTCGTTCA TCCATAGTTG CCTGACTCCC CGTCGTGTAG
4741  ATAACTACGA TACGGGAGGG CTTACCATCT GGCCCCAGTG CTGCAATGAT ACCGCGAGAC
4801  CCACGCTCAC CGGCTCCAGA TTTATCAGCA ATAAACCAGC CAGCCGGAAG GGCCGAGCGC
4861  AGAAGTGGTC CTGCAACTTT ATCCGCCTCC ATCCAGTCTA TTAATTGTTG CCGGGAAGCT
4921  AGAGTAAGTA GTTCGCCAGT TAATAGTTTG CGCAACGTTG TTGCCATTGC TGCAGGCATC
4981  GTGGTGTCAC GCTCGTCGTT TGGTATGGCT TCATTCAGCT CCGGTTCCCA ACGATCAAGG
5041  CGAGTTACAT GATCCCCCAT GTTGTGCAAA AAAGCGGTTA GCTCCTTCGG TCCTCCGATC
5101  GTTGTCAGAA GTAAGTTGGC CGCAGTGTTA TCACTCATGG TTATGGCAGC ACTGCATAAT
5161  TCTCTTACTG TCATGCCATC CGTAAGATGC TTTTCTGTGA CTGGTGAGTA CTCAACCAAG
5221  TCATTCTGAG AATAGTGTAT GCGGCGACCG AGTTGCTCTT GCCCGGCGTC AACACGGGAT
5281  AATACCGCGC CACATAGCAG AACTTTAAAA GTGCTCATCA TTGGAAAACG TTCTTCGGGG
5341  CGAAAACTCT CAAGGATCTT ACCGCTGTTG AGATCCAGTT CGATGTAACC CACTCGTGCA
5401  CCCAACTGAT CTTCAGCATC TTTTACTTTC ACCAGCGTTT CTGGGTGAGC AAAAACAGGA
5461  AGGCAAAATG CCGCAAAAAA GGGAATAAGG GCGACACGGA AATGTTGAAT ACTCATACTC
5521  TTCCTTTTTC AATATTATTG AAGCATTTAT CAGGGTTATT GTCTCATGAG CGGATACATA
5581  TTTGAATGTA TTTAGAAAAA TAAACAAATA GGGGTTCCGC GCACATTTCC CCGAAAAGTG
5641  CCACCTGACG TCTAAGAAAC CATTATTATC ATGACATTAA CCTATAAAAA TAGGCGTATC
5701  ACGAGGCCCT TTCGTCTTCA AGAATTCATA CCAGATCACC GAAAACTGTC CTCCAAATGT
5761  GTCCCCCTCA CACTCCCAAA TTCGCGGGCT TCTGCCTCTT AGACCACTCT ACCCTATTCC
5821  CCACACTCAC CGGAGCCAAA GCCGCGGCCC TTCCGTTTCT TTGCT
```

What is claimed is:

1. A method of treating a patient for a T-cell mediated autoimmune disease comprising:

introducing DNA comprising a sequence encoding one or more antigenic proteins into the cells of said patient, said cells expressing in said patient a therapeutically effective amount of said antigenic protein or proteins to restore T-cell tolerance to said patient.

2. The process of claim 1 wherein said patient is human.

3. The process of claim 2 wherein said cells are fibroblast cells.

4. The process of claim 3 wherein said fibroblast cells are histocompatible.

5. The process of claim 2 wherein said DNA encodes an amino acid sequence derived from a nervous system protein.

6. The process of claim 2 wherein the disease is multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, psoriasis, juvenile onset diabetes, Sjogren's disease, thyroid disease, myasthenia gravis, or chronic inflammatory demyelinating polyneuropathy (CIDP).

7. The method of claim 1 wherein the disease is multiple sclerosis.

8. A method of treating a human for a T-cell mediated auto immune disease comprising:

introducing DNA comprising a sequence encoding one or more amino acid sequence derived from a self-antigenic protein into the cells of said human, said cells expressing in said human a therapeutically effective amount of said self-antigenic protein or proteins to restore T-cell tolerance to said human.

9. A method of treating a human for multiple sclerosis comprising:

introducing DNA comprising a sequence encoding one or more amino acid sequence derived from a nervous system protein into the cells of said human, said cells secreting in said human a therapeutically effective amount of said self-antigenic protein or proteins to restore T-cell tolerance to said human.

10. A method of treating a patient for a T-cell mediated autoimmune disease comprising:

introducing mammalian cells into a patient, said cells having been treated in vitro to insert therein a DNA segment encoding one or more antigenic protein, said mammalian cells expressing in vivo in said patient a therapeutically effective amount of said antigenic protein or proteins to restore T-cell tolerance to said patient.

11. The process of claim 10 wherein said patient is human.

12. The process of claim 11 wherein said cells are fibroblast cells.

13. The process of claim 12 wherein said fibroblast cells are histocompatible.

14. The process of claim 11 wherein said disease is multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, psoriasis, juvenile onset diabetes, Sjogren's disease, thyroid disease, or myasthenia gravis or chronic inflammatory demyelinating polyneuropathy (CIDP).

15. The process of claim 10 wherein said disease is multiple sclerosis.

16. The process of claim 10 wherein said DNA segment has been inserted into said cells in vitro by a recombinant vector.

17. The process of claim 10 wherein said DNA segment has been inserted into said cells in vitro by a viral vector.

18. The process of claim 17 wherein said viral vector is a retroviral vector.

19. The process of claim 10 wherein said DNA segment encodes a protein comprising an amino acid sequence derived from a nervous system self-antigenic protein.

20. The process of claim 11 wherein said DNA segment encodes a protein comprising an amino acid sequence derived from a nervous system self-antigenic protein.

21. The process of claim 14 wherein said DNA segment encodes a protein comprising an amino acid sequence derived from a self-antigenic protein.

22. The process of claim 12 wherein said DNA segment encodes a protein comprising an amino acid sequence derived from a nervous system protein.

23. The process of claim 13 wherein said DNA segment encodes a protein comprising an amino acid sequence derived from a nervous system protein.

24. The process of claim 15 wherein said DNA segment encodes a protein comprising an amino acid sequence derived from a nervous system protein.

25. The process of claim 16 wherein said DNA segment encodes a protein comprising an amino acid sequence derived from a nervous system protein.

25. The process of claim 17 wherein the DNA segment encodes a protein comprising an amino acid sequence derived from a nervous system protein.

26. The process of claim 18 wherein the DNA segment encodes a protein comprising an amino acid sequence derived from a nervous system protein.

28. The process of claim 10 wherein said DNA segment encodes a protein comprising an amino acid sequence derived from a nervous system protein selected from the group consisting of myelin basic protein, proteolipid protein, and myelin-oligodendrocyte glycoprotein.

29. The process of claim 11 wherein said DNA segment encodes a protein comprising an amino acid sequence derived from a nervous system protein selected from the group consisting of myelin basic protein, proteolipid protein, and myelin-oligodendrocyte glycoprotein.

30. The process of claim 12 wherein said DNA segment encodes a protein comprising an amino acid sequence derived from a nervous system protein selected from the group consisting of myelin basic protein, proteolipid protein, and myelin-oligodendrocyte glycoprotein.

31. The process of claim 13 wherein said DNA segment encodes a protein comprising an amino acid sequence derived from a nervous system protein

selected from the group consisting of myelin basic protein, proteolipid protein, and myelin-oligodendrocyte glycoprotein.

32. The process of claim 15 wherein said DNA segment encodes a protein comprising an amino acid sequence derived from a nervous system protein selected from the group consisting of myelin basic protein, proteolipid protein, and myelin-oligodendrocyte glycoprotein.

33. The process of claim 16 wherein said DNA segment encodes a protein comprising an amino acid sequence derived from a nervous system protein selected from the group consisting of myelin basic protein, proteolipid protein, and myelin-oligodendrocyte glycoprotein.

34. The process of claim 17 wherein said DNA segment encodes a protein comprising an amino acid sequence derived from a nervous system protein selected from the group consisting of myelin basic protein, proteolipid protein, and myelin-oligodendrocyte glycoprotein.

35. The process of claim 18 wherein said DNA segment encodes a protein comprising an amino acid sequence derived from a nervous system protein selected from the group consisting of myelin basic protein, proteolipid protein, and myelin-oligodendrocyte glycoprotein.

36. The process of claim 10 wherein said DNA segment encodes a protein comprising an encephalitogenic epitope selected from the group consisting of encephalitogenic epitopes of myelin basic protein, encephalitogenic epitopes of myelin-oligodendrocyte glycoprotein, and encephalitogenic epitopes of proteolipid protein.

37. The process of claim 11 wherein said DNA segment encodes a protein comprising an encephalitogenic epitope selected from the group consisting of encephalitogenic epitopes of myelin basic protein, encephalitogenic epitopes of myelin-oligodendrocyte glycoprotein, and encephalitogenic epitopes of proteolipid protein.

38. The process of claim 12 wherein said DNA segment encodes a protein comprising an encephalitogenic epitope selected from the group consisting of encephalitogenic epitopes of myelin basic protein, encephalitogenic epitopes of myelin-oligodendrocyte glycoprotein, and encephalitogenic epitopes of proteolipid protein.

39. The process of claim 13 wherein said DNA segment encodes a protein comprising an encephalitogenic epitope selected from the group consisting of encephalitogenic epitopes of myelin basic protein, encephalitogenic epitopes of myelin-oligodendrocyte glycoprotein, and encephalitogenic epitopes of proteolipid protein.

40. The process of claim 15 wherein said DNA segment encodes an protein comprising an encephalitogenic epitope selected from the group consisting of encephalitogenic epitopes of myelin basic protein, encephalitogenic epitopes of myelin-oligodendrocyte glycoprotein, and encephalitogenic epitopes of proteolipid protein.

41. The process of claim 16 wherein said DNA segment encodes an protein comprising an encephalitogenic epitope selected from the group consisting of encephalitogenic epitopes of myelin basic protein, encephalitogenic epitopes of myelin-oligodendrocyte glycoprotein, and encephalitogenic epitopes of proteolipid protein.

42. The process of claim 17 wherein said DNA segment encodes a protein comprising an encephalitogenic epitope selected from the group consisting of encephalitogenic epitopes of myelin basic protein, encephalitogenic epitopes of myelin-oligodendrocyte glycoprotein, and encephalitogenic epitopes of proteolipid protein.

43. The process of claim 18 wherein said DNA segment encodes a protein comprising an encephalitogenic epitope selected from the group consisting of encephalitogenic epitopes of myelin basic protein, encephalitogenic epitopes of myelin-oligodendrocyte glycoprotein, and encephalitogenic epitopes of proteolipid protein.

44. The process of any one of claims 19-43 wherein said DNA segment additionally comprises a hydrophobic leader sequence, said hydrophobic leader sequence enabling the gene product to be synthesized in an endoplasmic reticulum for later constitutive secretion.

45. The process of any one of claims 19-43 wherein said DNA segment further comprises a Kozak box, said Kozak box permitting efficient translation of an mRNA transcribed from said DNA segment.

46. The process of any one of claims 19-43 wherein said DNA segment further comprises a codon corresponding to a charged amino acid at the 3' end to ensure that the protein is not retained in membrane.

47. The process of any one of claims 19-43 wherein said DNA segment further comprises one or more restriction sites to permit insertion of additional gene sequences.

48. The process of any one of claims 19-43 wherein said DNA sequence encodes amino acids 101-157 of proteolipid protein.

49. A method of treating a human patient for multiple sclerosis comprising:

introducing mammalian cells into said human patient, said mammalian cells having been treated in vitro to insert therein a DNA segment encoding one or

more encephalitogenic epitope derived from nervous system protein, said mammalian cells expressing in vivo in said human patient a therapeutically effective amount of said encephalitogenic epitope or epitopes to restore T-cell tolerance to said human patient.

50. The method of claim 49 wherein said mammalian cells are fibroblasts cells.

51. The method of claim 50 wherein said mammalian fibroblast cells are histocompatible.

52. A method of treating a human patient for multiple sclerosis comprising:

introducing histocompatible fibroblast cells into said human patient, said histocompatible fibroblast cells having been treated in vitro to insert therein a DNA segment encoding amino acids 101-157 of proteolipid protein, said DNA segment introduced into said histocompatible fibroblasts cells in vitro by a recombinant retroviral vector, said DNA sequence comprising a hydrophobic leader sequence whereby said leader sequence enables said amino acids 101-157 of proteolipid protein to be synthesized in the endoplasmic reticulum of said histocompatible fibroblast cells for later constitutive secretion, said DNA segment further comprising a Kozak box permitting efficient translation of mRNA transcribed from said DNA segment, said DNA segment further comprising a codon corresponding to a charged amino acid at the 3' end to ensure that the protein is not retained in membrane, said DNA segment further

comprising one or more restriction sites to permit insertion of additional gene sequences, whereby the gene product or gene products of said DNA segment is expressed in said human in a therapeutically effective amount to restore T-cell tolerance to said human.

53. An engineered cell comprising a gene encoding one or more antigenic protein which can be expressed, wherein said gene has been introduced into the cell by means of a recombinant vector.

54. An engineered cell comprising a gene encoding one or more antigenic protein which can be secreted, wherein said gene has been introduced into the cell by means of a recombinant vector.

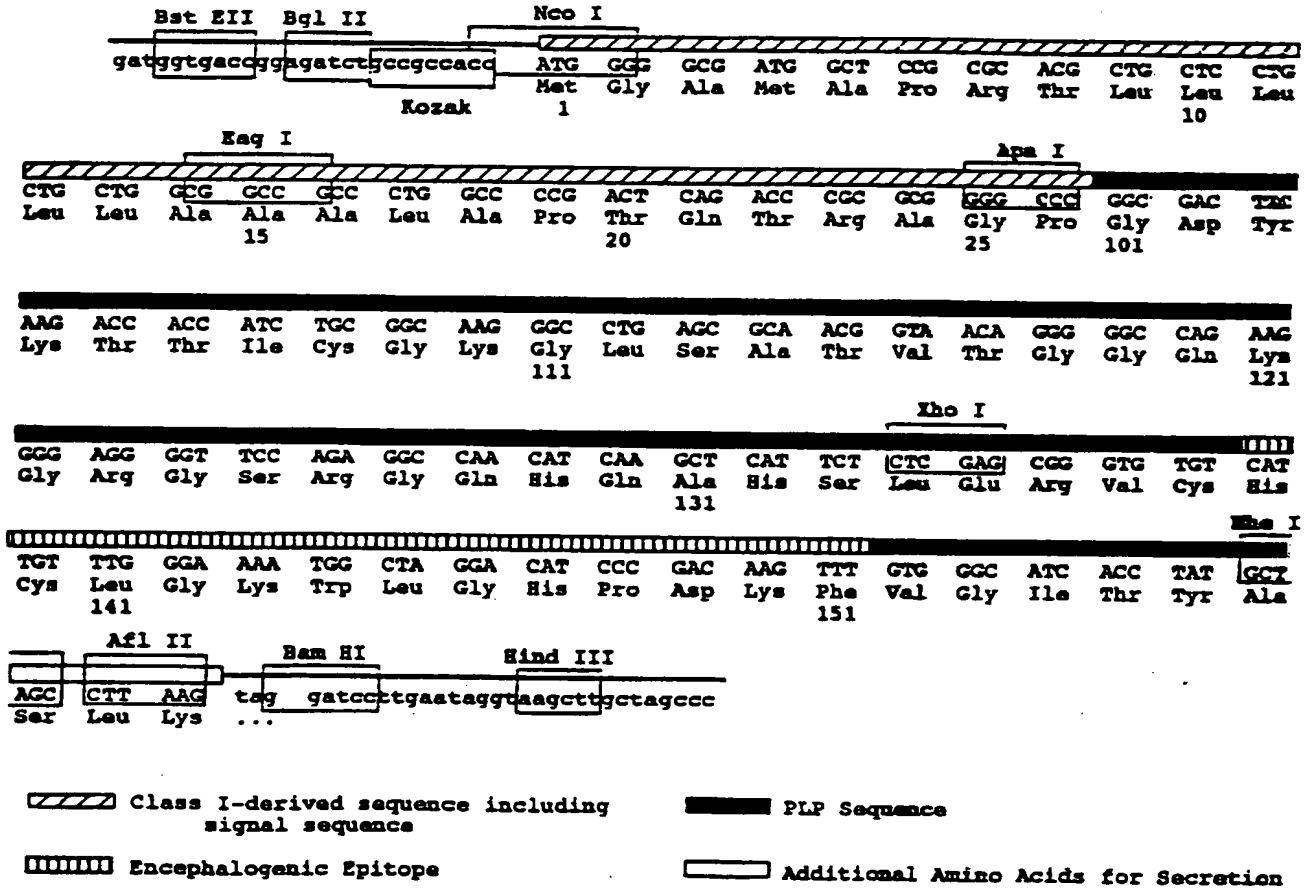
55. An engineered cell comprising a gene encoding one or more encephalitogenic epitope which can be expressed, wherein said gene has been introduced into the cell by means of a recombinant vector.

56. An engineered cell comprising a gene encoding one or more encephalitogenic epitope which can be secreted, wherein said gene has been introduced into the cell by means of a recombinant vector.

57. Any one of claims 53-56 wherein said recombinant vector is a retroviral vector.

58. The cell of claim 56 wherein said gene comprises the sequence encoding amino acids 101-157 of proteolipid protein.

FIGURE 1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Bgl II
Nco I
Kozak
Met 1
Gly
Ala
Met
Ala
Pro
Arg
Thr
Leu
Leu 10
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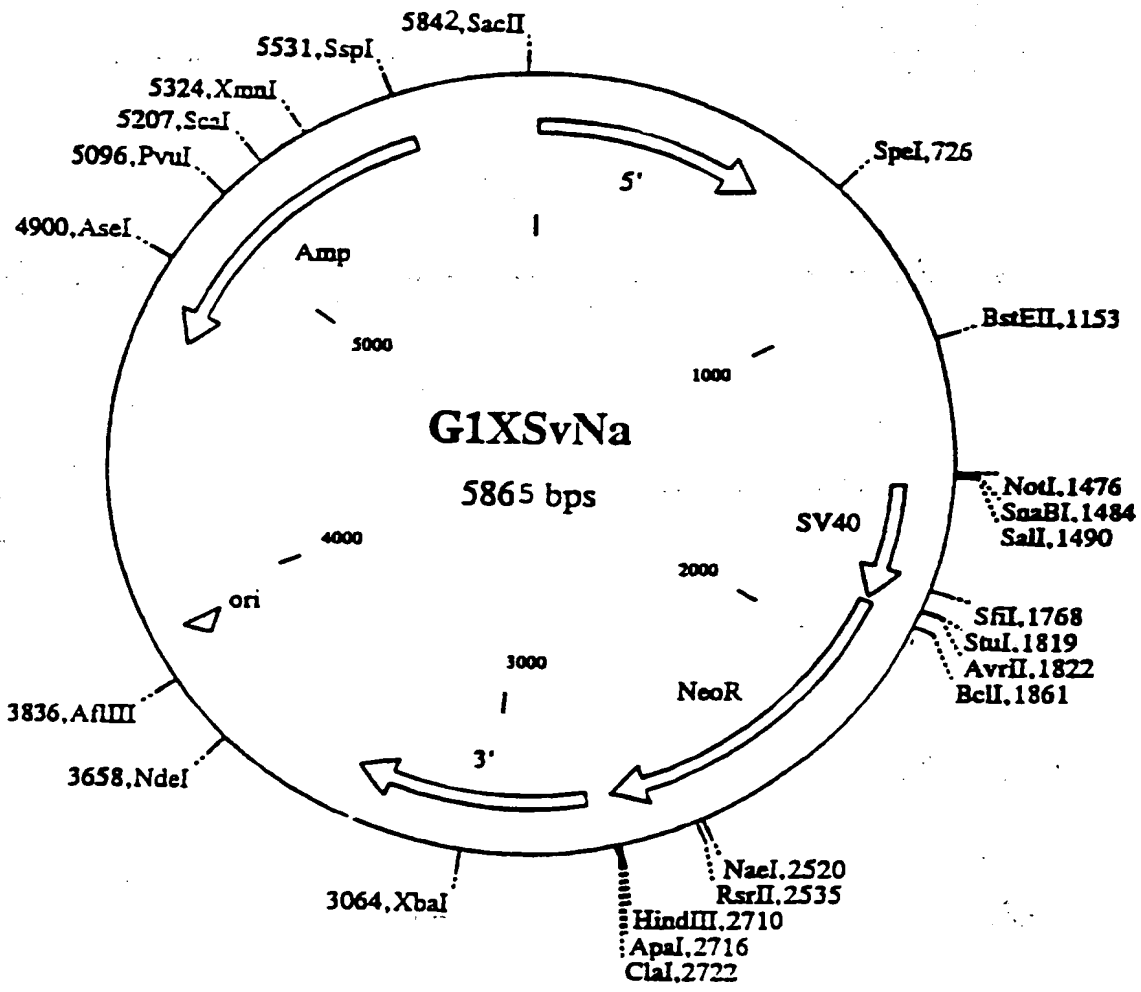
Eag I
Apa I
Ala 15
Thr 20
Gln
Thr
Arg
Ala
Gly 25
Pro
Gly 101
Asp
Tyr
Lys
Thr
Thr
Ile
Cys
Gly
Lys
Gly 111
Leu
Ser
Ala
Thr
Val
Thr
Gly
Gly
Gln
Lys 121
Xho I
Gly
Arg
Gly
Ser
Arg
Gly
Gln
His
Gln
Ala 131
His
Ser
Leu
Glu
Arg
Val
Cys
His
Xho I
Cys
Leu 141
Gly
Lys
Trp
Leu
Gly
His
Pro
Asp
Lys
Phe 151
Val
Gly
Ile
Thr
Tyr
Ala
Afl II
Bam HI
Hind III
Ser
Leu
Lys
Class I-derived sequence including signal sequence
PLP Sequence
Encephalogenic Epitope
Additional Amino Acids for Secretion

Figure 2A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5531,SspI
5324,XmnI
5207,ScaI
5096,PvuI
4900,AseI
SpeI,726
BstEII,1153
NotI,1476
SnaBI,1484
SalI,1490
SfiI,1768
StuI,1819
AvrII,1822
BclI,1861
NaeI,2520
RsrII,2535
HindIII,2710
ApaI,2716
ClaI,2722
3064,XbaI
3658,NdeI
3836,AflIII
ori
Amp
SV40
NeoR
5'
3'
1000
2000
3000
4000
5000
G1XSvNa
5865 bps

FIGURE 2B

NAME: G1XSvNa 5865 BPS DNA CIRCULAR 27-FEB-1996
DESCRIPTION: Sal/Hind frag of pSVNA into Sal/Hind cut G1

* * * S E Q U E N C E * * *

```
   1 TTTGAAAGAC CCCACCCGTA GGTGGCAAGC TAGCTTAAGT AACGCCACTT TGCAAGGCAT
  61 GGAAAAATAC ATAACTGAGA ATAGAAAAGT TCAGATCAAG GTCAGGAACA AAGAAACAGC
 121 TGAATACCAA ACAGGATATC TGTGGTAAGC GGTTCCTGCC CCGGCTCAGG GCCAAGAACA
 181 GATGAGACAG CTGAGTGATG GGCCAAACAG GATATCTGTG GTAAGCAGTT CCTGCCCCGG
 241 CTCGGGGCCA AGAACAGATG GTCCCCAGAT GCGGTCCAGC CCTCAGCAGT TTCTAGTGAA
 301 TCATCAGATG TTTCCAGGGT GCCCCAAGGA CCTGAAAATG ACCCTGTACC TTATTTGAAC
 361 TAACCAATCA GTTCGCTTCT CGCTTCTGTT CGCGCGCTTC CGCTCTCCGA GCTCAATAAA
 421 AGAGCCCACA ACCCCTCACT CGGCGCGCCA GTCTTCCGAT AGACTGCGTC GCCCGGGTAC
 481 CCGTATTCCC AATAAAGCCT CTTGCTGTTT GCATCCGAAT CGTGGTCTCG CTGTTCCTTG
 541 GGAGGGTCTC CTCTGAGTGA TTGACTACCC ACGACGGGGG TCTTTCATTT GGGGGCTCGT
 601 CCGGGATTTG GAGACCCCTG CCCAGGGACC ACCGACCCAC CACCGGGAGG TAAGCTGGCC
 661 AGCAACTTAT CTGTGTCTGT CCGATTGTCT AGTGTCTATG TTTGATGTTA TGCGCCTGCG
 721 TCTGTACTAG TTAGCTAACT AGCTCTGTAT CTGGCGGACC CGTGGTGGAA CTGACGAGTT
 781 CTGAACACCC GGCCGCAACC CTGGGAGACG TCCCAGGGAC TTTGGGGGCC GTTTTTGTGG
 841 CCCGACCTGA GGAAGGGAGT CGATGTGGAA TCCGACCCCG TCAGGATATG TGGTTCTGGT
 901 AGGAGACGAG AACCTAAAAC AGTTCCCGCC TCCGTCTGAA TTTTTGCTTT CGGTTTGGAA
 961 CCGAAGCCGC GCGTCTTGTC TGCTGCAGCG CTGCAGCATC GTTCTGTGTT GTCTCTGTCT
1021 GACTGTGTTT CTGTATTTGT CTGAAAATTA GGGCCAGACT GTTACCACTC CCTTAAGTTT
1081 GACCTTAGGT CACTGGAAAG ATGTCGAGCG GATCGCTCAC AACCAGTCGG TAGATGTCAA
1141 GAAGAGACGT TGGGTTACCT TCTGCTCTGC AGAATGGCCA ACCTTTAACG TCGGATGGCC
1201 GCGAGACGGC ACCTTTAACC GAGACCTCAT CACCCAGGTT AAGATCAAGG TCTTTTCACC
1261 TGGCCCGCAT GGACACCCAG ACCAGGTCCC CTACATCGTG ACCTGGGAAG CCTTGGCTTT
1321 TGACCCCCCT CCCTGGGTCA AGCCCTTTGT ACACCCTAAG CCTCCGCCTC CTCTTCCTCC
1381 ATCCGCCCCG TCTCTCCCCC TTGAACCTCC TCGTTCGACC CCGCCTCGAT CCTCCCTTTA
1441 TCCAGCCCTC ACTCCTTCTC TAGGCGCCGG AATTCGCGGC CGCTACGTAG TCGACTCGCT
1501 GTGGAATGTG TGTCAGTTAG GGTGTGGAAA GTCCCCAGGC TCCCCAGCAG GCAGAAGTAT
1561 GCAAAGCATG CATCTCAATT AGTCAGCAAC CAGGTGTGGA AAGTCCCCAG GCTCCCCAGC
1621 AGGCAGAAGT ATGCAAAGCA TGCATCTCAA TTAGTCAGCA ACCATAGTCC CGCCCCTAAC
1681 TCCGCCCATC CCGCCCCTAA CTCCGCCCAG TTCCGCCCAT TCTCCGCCCC ATGGCTGACT
1741 AATTTTTTTT ATTTATGCAG AGGCCGAGGC CGCCTCGGCC TCTGAGCTAT TCCAGAAGTA
1801 GTGAGGAGGC TTTTTTGGAG GCCTAGGCTT TTGCAAAAAG CTCGAAGATC AATTCCGATC
1861 TGATCAAGAG ACAGGATGAG GATCGTTTCG CATGATTGAA CAAGATGGAT TGCACGCAGG
1921 TTCTCCGGCC GCTTGGGTGG AGAGGCTATT CGGCTATGAC TGGGCACAAC AGACAATCGG
1981 CTGCTCTGAT GCCGCCGTGT TCCGGCTGTC AGCGCAGGGG CGCCCGGTTC TTTTTGTCAA
2041 GACCGACCTG TCCGGTGCCC TGAATGAACT GCAGGACGAG GCAGCGCGGC TATCGTGGCT
2101 GGCCACGACG GGCGTTCCTT GCGCAGCTGT GCTCGACGTT GTCACTGAAG CGGGAAGGGA
2161 CTGGCTGCTA TTGGGCGAAG TGCCGGGGCA GGATCTCCTG TCATCTCACC TTGCTCCTGC
2221 CGAGAAAGTA TCCATCATGG CTGATGCAAT GCGGCGGCTG CATACGCTTG ATCCGGCTAC
2281 CTGCCCATTC GACCACCAAG CGAAACATCG CATCGAGCGA GCACGTACTC GGATGGAAGC
2341 CGGTCTTGTC GATCAGGATG ATCTGGACGA AGAGCATCAG GGGCTCGCGC CAGCCGAACT
2401 GTTCGCCAGG CTCAAGGCGC GCATGCCCGA CGGCGAGGAT CTCGTCGTGA CCCATGGCGA
2461 TGCCTGCTTG CCGAATATCA TGGTGGAAAA TGGCCGCTTT TCTGGATTCA TCGACTGTGG
2521 CCGGCTGGGT GTGGCGGACC GCTATCAGGA CATAGCGTTG GCTACCCGTG ATATTGCTGA
2581 AGAGCTTGGC GGCGAATGGG CTGACCGCTT CCTCGTGCTT TACGGTATCG CCGCTCCCGA
2641 TTCGCAGCGC ATCGCCTTCT ATCGCCTTCT TGACGAGTTC TTCTGAGCGG GACTCTGGGG
2701 TTCGTCGAGA AGCTTGGGCC CATCGATAAA ATAAAAGATT TTATTTAGTC TCCAGAAAAA
2761 GGGGGGAATG AAAGACCCCA CCTGTAGGTT TGGCAAGCTA GCTTAAGTAA CGCCATTTTG
2821 CAAGGCATGG AAAAATACAT AACTGAGAAT AGAGAAGTTC AGATCAAGGT CAGGAACAGA
2881 TGGAACAGCT GAATATGGGC CAAACAGGAT ATCTGTGGTA AGCAGTTCCT GCCCCGGCTC
2941 AGGGCCAAGA ACAGATGGAA CAGCTGAATA TGGGCCAAAC AGGATATCTG TGGTAAGCAG
3001 TTCCTGCCCC GGCTCAGGGC CAAGAACAGA TGGTCCCCAG ATGCGGTCCA GCCCTCAGCA
3061 GTTTCTAGAG AACCATCAGA TGTTTCCAGG GTGCCCCAAG GACCTGAAAT GACCCTGTGC
3121 CTTATTTGAA TTAACCAATC AGTTCGCTTC TCGCTTCTGT TCGCGCGCTT CTGCTCCCCG
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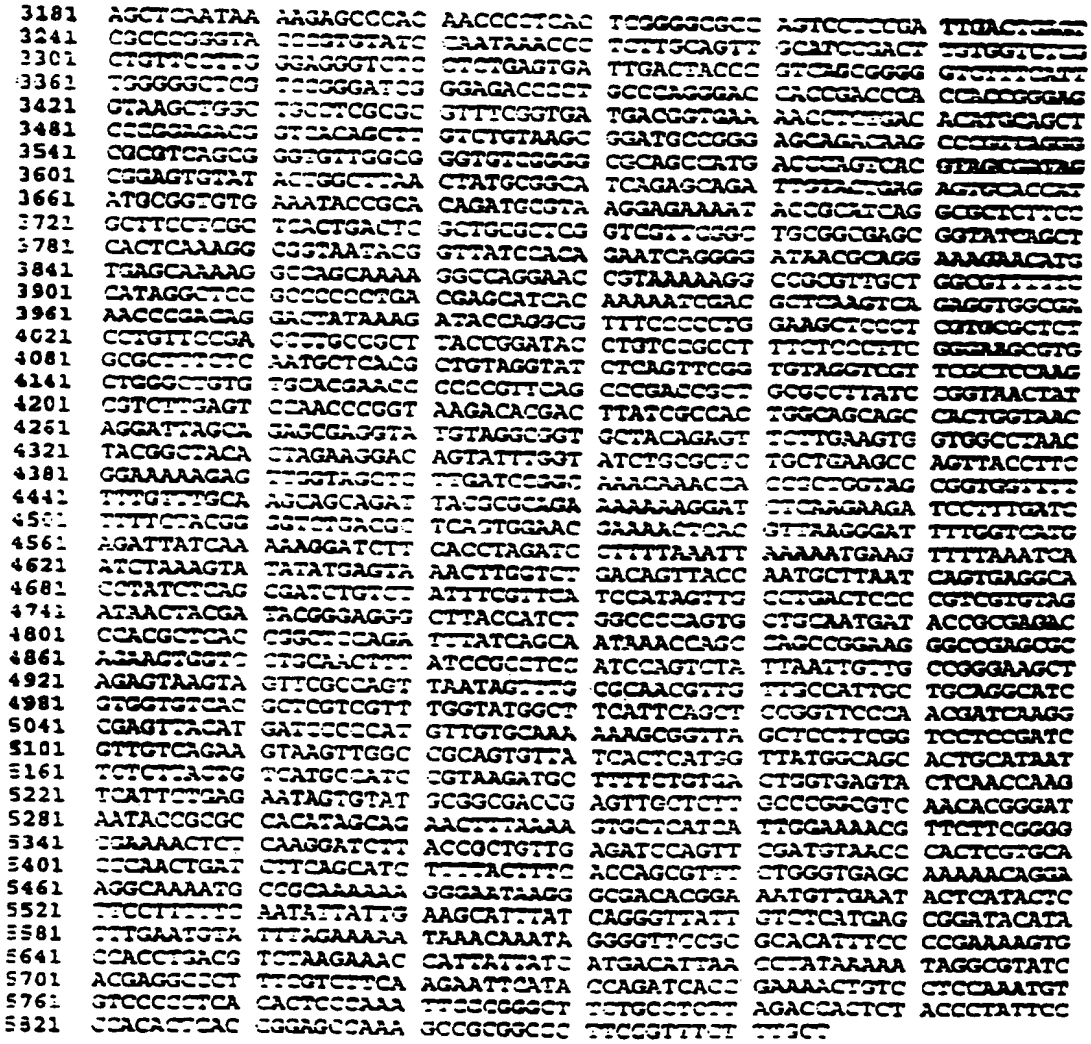
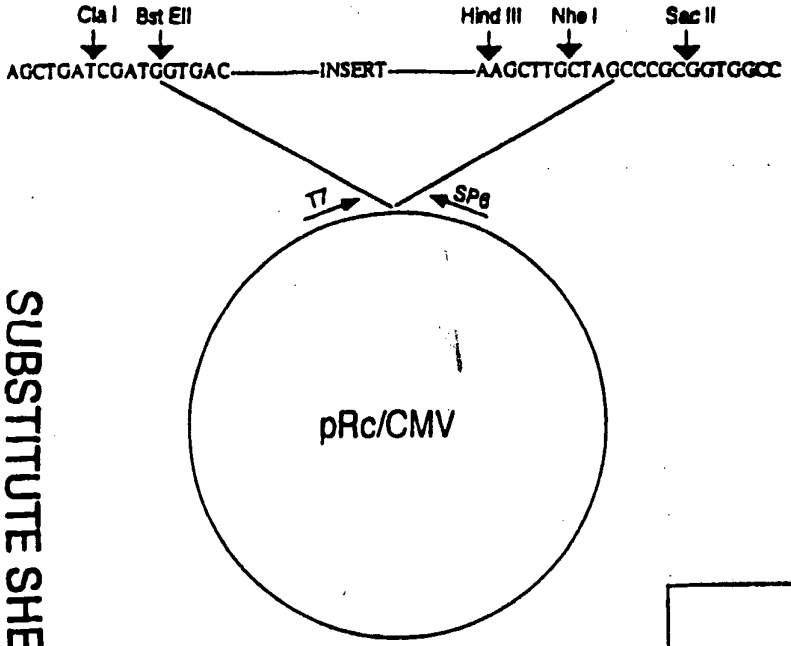
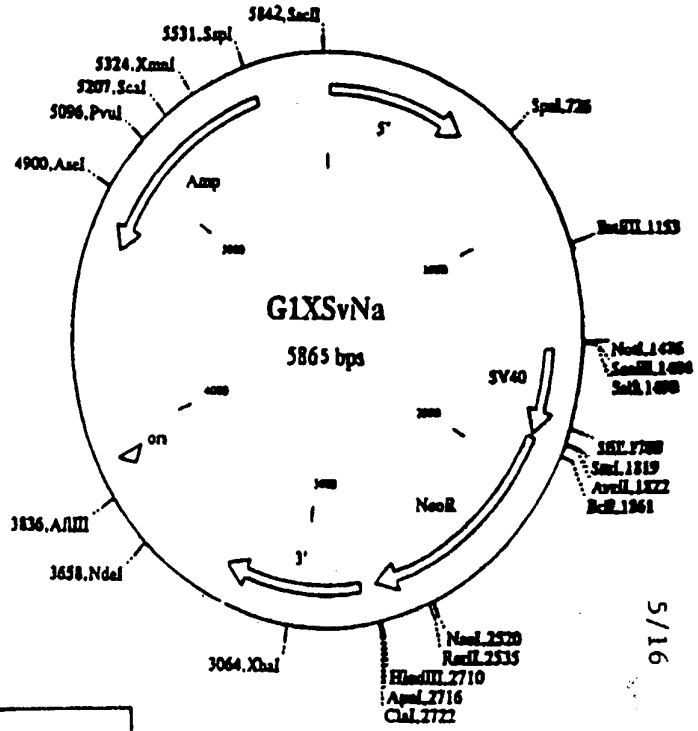


FIGURE 2B continued

pRC/CMV's original multiple cloning site (between Hind III and Apa I) was replaced with a new multiple cloning site/PLP insert. The original Hind III and Apa I sites were destroyed.





BstEII/HindIII Digest

↓

Gel isolate

↓

Ligate HindIII/NotI adapters

↓

NotI digest

↓

Gel Isolate

Ligate NotI insert site into NotI vector

↓

Klenow unligated ends

↓

Blunt end ligation

↓

Transform HB101

↓

Restriction analysis of transformants

↓

Sequence

NotI Digest

↓

CIAP

↓

Gel Isolate

Figure 3

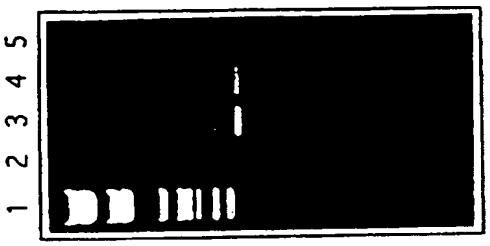


Figure 4. mRNA expression levels as detected by reverse transcriptase PCR.

FIGURE 5

Sample	OD of reaction product measured at 490 nm
1. PLP peptide 139-151	1.06±0.41
2. HIV gp120 peptide control	0.10±0.09
3. Supernatant of SJL fibroblasts transduced with PLP retrovirus. Sample I	0.92±0.50
4. Supernatant of SJL cultured fibroblasts transduced with PLP retrovirus. Sample II	0.73±0.17
5. Supernatant of cultured fibroblasts transduced with ß-galactosidase (LacZ) construct	0.05±0.01

1. PLP and HIV gp120 peptides used at a concentration of 5 ug/ml.
2. All supernatants used undiluted.
3. Primary monoclonal antibody was used as an undiluted hybridoma supernatant.
4. Peroxidase conjugated secondary goat anti-antibody used at a dilution 1:500.

Figure 5. ELISA assays on transduced fibroblast supernatants

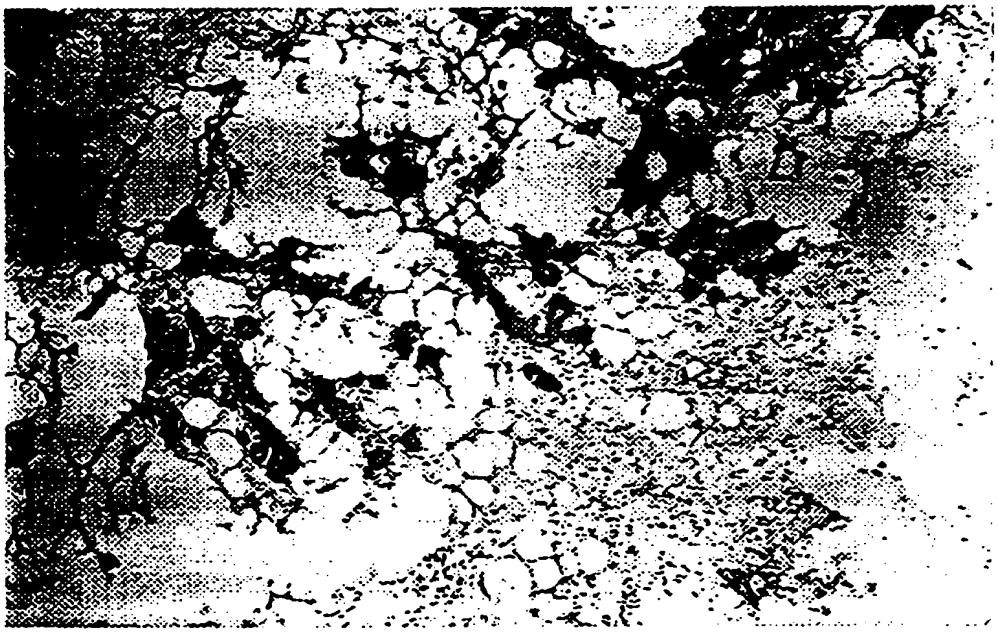


Figure 6. B-Gal expression in transduced fibroblasts.

FIGURE 7

Grade 0	no abnormality
Grade 1	slow, sluggish
Grade 2	limp tail
Grade 3	limp tail, hand/limb weakness, waddling gait
Grade 4	partial hind limb paralysis
Grade 5	complete hind limb paralysis
Grade 6	animal immobile
Grade 7	moribund

Figure 7. Clinical Scores Chronic EAE

FIGURE 8

Histological Scoring System

1+ **mild** (1-3 small foci)

2+ **moderate** (more than 3-7 foci containing at least 10)

3+ **severe** (large foci of 15 to 25 cells with perivascular and meningeal collections)

4+ **severe with necrosis and demyelination**

FIGURE 9

		Day 55-60					Day 90-95				
Grade	# Animals	A 40	B 28	C 38	D 30	E 32	A 17	B 13	C 8	D 14	E 18
0		2	2	0	1	30	0	2	0	0	17
1		2	0	0	1	1	1	0	4	1	0
2		14	10	20	8	0	0	0	4	8	0
3		14	11	11	3	0	10	11	0	4	0
4		5	1	2	2	1*	4	0	0	1	1*
5		0	0	1	2	0	0	0	0	0	0
6		3	4	4	0	0	3	0	0	0	0
7		0	0	0	0	0	0	0	0	0	0

*Paralysis with first relapse no further progression.

A = EAE control; B = EAE + untransduced fibroblasts; C = EAE + fibroblasts with Lac-Z retrovirus; D = EAE + neo fibroblasts with neo-retrovirus; E = EAE + PLP fibroblasts with PLP-retrovirus.

Figure 9. Clinical assessment of EAE mice treated with retrovirus transduced fibroblasts

PATHOLOGIC ASSESSMENT OF BRAIN AND SPINAL CORD

FIGURE 10A

	Day 55-60					Day 90-95				
	0	+	++	+++	++++	0	+	++	+++	++++
EAE Control	1	2	2	1	2	0	0	0	3	5
EAE Untransduced fibroblasts	0	3	0	2	3	1	2	1	2	2
EAE Neoretrovirus	2	2	0	3	1	0	1	3	2	1
EAE LacZretrovirus	2	1	1	1	3	0	0	4	1	2
EAE PLPretrovirus	7	0	1	0	0	5	1	1	0	1*

*paralyzed animal

FIGURE 10B

Pathologic Assessment of Brain and

Spinal Cords from Days 55-60

through Days 90-95

TREATMENT DAY 21	Score 2+ or more
EAE Control	13/16
Untransduced fibroblasts	10/16
Neo-retrovirus	10/15
LacZ-retrovirus	12/15
PLP-retrovirus	3/16

FIGURE 11

Mouse	Score 0	+	++	+++	++++	Total (Score 2+ or more)
EAE Control	0	1	2	0	1	3/4
Untranduced fibroblasts	0	3	6	4	1	11/14
Neo-retrovirus	0	5	3	4	0	7/12
LacZ-retrovirus	0	2	7	3	0	10/12
PLP-retrovirus	11	3	0	0	0	0/14

Figure 11. Histology of EAE mice treated with retrovirus transduced fibroblasts

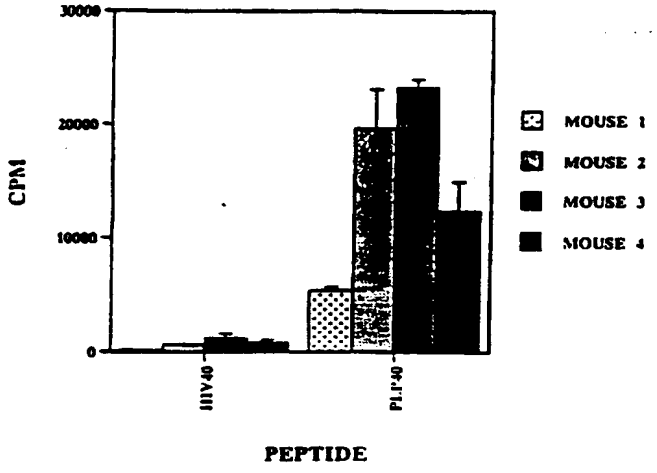


Figure 12A

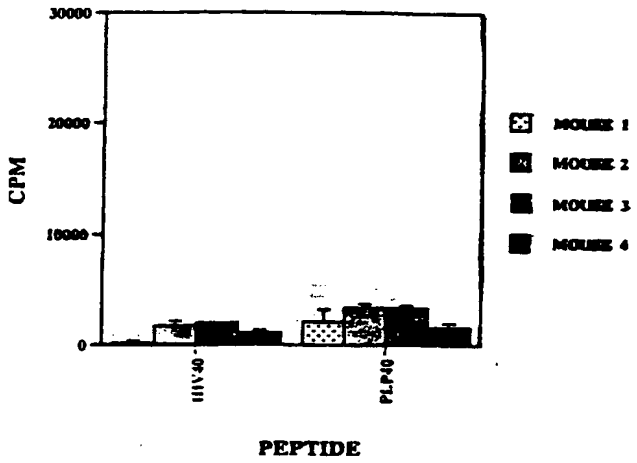


Figure 12 B

Proliferation assays using A) four control EAE mice and B) four EAE mice treated with PLP expressing fibroblasts

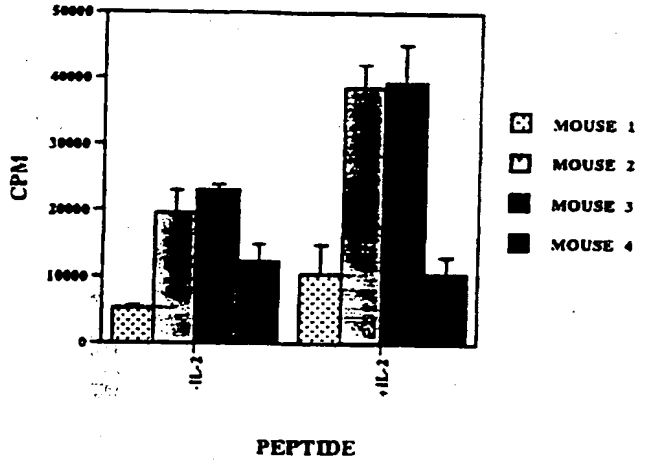


Figure 13A

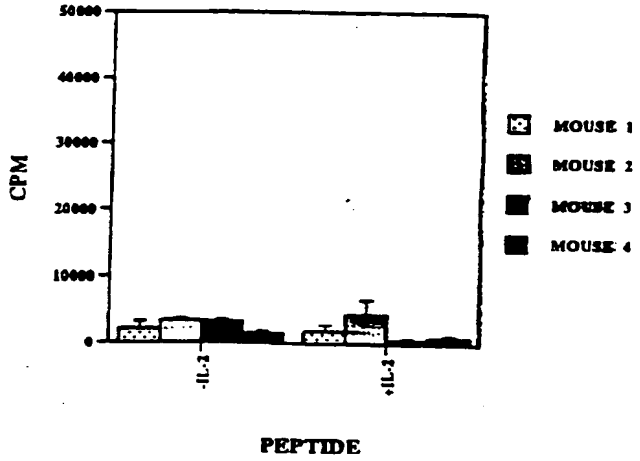


Figure 13 B

Proliferation assays in the absence (left-hand historgram) and presence (right-hand histogram) of IL-2 using A) four EAE control mice and B) four EAE mice treated with PLP-expressing fibroblasts

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/10214

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 48/00; C12N 5/00, 15/63
US CL :514/44; 424 93.21; 435/375, 172.3, 69.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/44; 424 93.21; 435/375, 172.3, 69.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN, MEDLINE, BIOSIS, APS
search terms: autoimmune, myelin, protein, tolerance, therapy

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	AL-SABBAGH et al. Antigen-driven tissue-specific suppression following oral tolerance: orally administered myelin basic protein suppresses proteolipid protein-induced experimental autoimmune encephalomyelitis in the SJL mouse. Eur. J. Immunology. September 1994, Vol. 24, No. 9, pages 2104-2109, see entire document.	1-58
Y	JAVED et al. Exquisite peptide specificity of oral tolerance in experimental autoimmune encephalomyelitis. J. of Immunology. 01 August 1995, Vol. 155, No. 3, pages 1599-1605, see entire document.	1-58

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
09 SEPTEMBER 1997

Date of mailing of the international search report
18 SEP 1997

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/10214

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	YU et al. A predictable sequential determinant spreading cascade invariably accompanies progression of experimental autoimmune encephalomyelitis: a basis for peptide-specific therapy after onset of clinical disease. J. of Experimental Medicine. 01 April 1996, Vol. 183, No. 4, pages 1777-1788, see entire document.	1-58
Y	WRAITH, D.C. Induction of antigen-specific unresponsiveness with synthetic peptides: specific immunotherapy for treatment of allergic and autoimmune conditions. International Archives of Allergy and Immunology. December 1995, Vol. 108, No. 4, pages 355-359, see entire document.	1-58
Y	GAUR et al. Amelioration of autoimmune encephalomyelitis by myelin basic protein synthetic peptide-induced anergy. Science. 27 November 1992, Vol. 258, No. 5087, pages 1491-1494, see entire document.	1-58
Y, P	US 5,589,466 A (FELGNER et al.) 31 December 1996, entire patent, especially claims.	1-58
Y	US 5,264,618 A (FELGNER et al.) 23 November 1993, entire patent.	1-58
Y	US 5,459,127 A (FELGNER et al.) 17 October 1995, entire patent.	1-58
Y, P	US 5,580,859 A (FELGNER et al.) 03 December 1996, entire patent.	1-58
Y	US 5,399,346 A (ANDERSON et al.) 21 March 1995, entire patent.	1-58